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<b>(54) Title:</b> THERMITASE VARIANTS HAVING DECREASED ADSORPTION AND INCREASED HYDROLYSIS		
<b>(57) Abstract</b>  The present invention relates to Thermitase variants having a modified amino acid sequence of wild-type Thermitase amino acid sequence, the wild-type amino acid sequence comprising a first loop region, a second loop region, a third loop region, a fourth loop region, a fifth loop region and a sixth loop region; wherein the modified amino acid sequence comprises different amino acids than that occurring in wild-type Thermitase (i.e., substitution) at specifically identified positions in one or more of the loop regions whereby the Thermitase variant has decreased adsorption to, and increased hydrolysis of, an insoluble substrate as compared to the wild-type Thermitase. The present invention also relates to DNA sequences encoding such Thermitase variants. The present invention also relates to compositions comprising such Thermitase variants for cleaning a variety of surfaces.		

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## THERMITASE VARIANTS HAVING DECREASED ADSORPTION AND INCREASED HYDROLYSIS

### TECHNICAL FIELD

The present invention relates to novel enzyme variants useful in a variety of cleaning compositions, and DNA sequences encoding such enzyme variants.

### BACKGROUND

Enzymes make up the largest class of naturally occurring proteins. Each class of enzyme generally catalyzes (accelerates a reaction without being consumed) a different kind of chemical reaction. One class of enzymes known as proteases, are known for their ability to hydrolyze (break down a compound into two or more simpler compounds with the uptake of the H and OH parts of a water molecule on either side of the chemical bond cleaved) other proteins. This ability to hydrolyze proteins has been taken advantage of by incorporating naturally occurring and protein engineered proteases as an additive to laundry detergent preparations. Many stains on clothes are proteinaceous and wide-specificity proteases can substantially improve removal of such stains.

Unfortunately, the efficacy level of these proteins in their natural, bacterial environment, frequently does not translate into the relatively unnatural wash environment. Specifically, protease characteristics such as thermal stability, pH stability, oxidative stability and substrate specificity are not necessarily optimized for utilization outside the natural environment of the enzyme.

The amino acid sequence of the protease determines the characteristics of the protease. A change of the amino acid sequence of the protease may alter the properties of the enzyme to varying degrees, or may even inactivate the enzyme, depending upon the location, nature and/or magnitude of the change in the amino acid sequence. Several approaches have been taken to alter the wild-type amino acid sequence of proteases in an attempt to improve their properties, with the goal of increasing the efficacy of the protease in the wash environment. These approaches include altering the amino acid sequence to enhance thermal stability and to

improve oxidation stability under quite diverse conditions.

Despite the variety of approaches described in the art, there is a continuing need for new effective variants of proteases useful for cleaning a variety of surfaces.

#### Objects of the Present Invention

It is an object of the present invention to provide Thermitase enzyme variants having improved hydrolysis versus the wild-type of the enzyme.

It is also an object of the present invention to provide cleaning compositions comprising these subtilisin enzyme variants.

#### SUMMARY

The present invention relates to Thermitase variants having a modified amino acid sequence of wild-type Thermitase amino acid sequence, the wild-type amino acid sequence comprising a first loop region, a second loop region, a third loop region, a fourth loop region and a fifth loop region; wherein the modified amino acid sequence comprises different amino acids than that occurring in wild-type Thermitase (i.e., substitution) at specifically identified positions in one or more of the loop regions whereby the Thermitase variant has decreased adsorption to, and increased hydrolysis of, an insoluble substrate as compared to the wild-type Thermitase. The present invention also relates to DNA sequences encoding such Thermitase variants. The present invention also relates to compositions comprising such Thermitase variants for cleaning a variety of surfaces.

#### DESCRIPTION

##### I. Thermitase Variants

This invention pertains to subtilisin enzymes, in particular Thermitase, that have been modified by mutating the various nucleotide sequences that code for the enzyme, thereby modifying the amino acid sequence of the enzyme. The modified subtilisin enzymes (hereinafter, "Thermitase variants") of the present invention have decreased adsorption to and increased hydrolysis of an insoluble substrate as compared to the wild-type subtilisin. The present invention also pertains to DNA sequences encoding for such Thermitase variants.

The subtilisin enzymes of this invention belong to a class of enzymes known as proteases. A protease is a catalyst for the cleavage of peptide bonds. One type of protease is a serine protease. A serine protease is distinguished by the fact that there is an essential serine residue at the

active site.

The observation that an enzyme's rate of hydrolysis of soluble substrates increases with enzyme concentration is well documented. It would therefore seem plausible that for surface bound substrates, such as is encountered in many cleaning applications, the rate of hydrolysis would increase with increasing surface concentration. This has been shown to be the case. (Brode, P.F. III and D. S. Rauch, *LANGMUIR*, "Subtilisin BPN": Activity on an Immobilized Substrate", Vol. 8, pp. 1325-1329 (1992)). In fact, a linear dependence of rate upon surface concentration was found for insoluble substrates when the surface concentration of the enzyme was varied. (Rubingh, D. N. and M. D. Bauer, "Catalysis of Hydrolysis by Proteases at the Protein-Solution Interface." in *POLYMER SOLUTIONS, BLENDS AND INTERFACES*. Ed. by I. Noda and D. N. Rubingh. Elsevier, p. 464 (1992)). Surprisingly, when seeking to apply this principle in the search for variant proteases which give better cleaning performance, we did not find that enzymes which adsorb more give better performance. In fact, we surprisingly determined the opposite to be the case: decreased adsorption by an enzyme to a substrate resulted in increased hydrolysis of the substrate (i.e., better cleaning performance).

While not wishing to be bound by theory, it is believed that improved performance, when comparing one variant to another, is a result of the fact that enzymes which adsorb less are also less tightly bound and therefore more highly mobile on the surface from which the insoluble protein substrate is to be removed. At comparable enzyme solution concentrations, this increased mobility is sufficient to outweigh any advantage that is conferred by delivering a higher concentration of enzyme to the surface.

The mutations described herein are designed to change (i.e., decrease) the adsorption of the enzyme to surface-bound soils. In *Thermitase*, certain amino acids form exterior loops on the enzyme molecule. For purposes of discussion, these loops shall be referred to as first, second, third, fourth and fifth loop regions. Specifically, positions 66-73 form the first loop region; positions 103-115 form the second loop region; positions 134-141 form the third loop region; positions 162-171 form the fourth loop region; positions 191-195 form the fifth loop region; and positions 204-224 form the sixth loop region (position numbering analogous to positions in the amino acid sequence for wild-type subtilisin *Thermitase* (SEQ ID NO:1)).

It is believed that these loop regions play a significant role in the adsorption of the enzyme molecule to a surface-bound peptide, and specific mutations in one or more of these loop regions will have a significant effect on this adsorption. While not wishing to be bound by theory, it is believed that the loop regions are important to the adsorption of the Thermitase molecule for at least two reasons. First, the amino acids which comprise the loop regions can make close contacts with any surfaces to which the molecule is exposed. Second, the proximity of the loop regions to the active-site and binding pocket of the Thermitase molecule gives them a role in the catalytically productive adsorption of the enzyme to surface-bound substrates (peptides/protein soils).

As used herein, "variant" means an enzyme having an amino acid sequence which differs from that of wild-type.

As used herein, "mutant Thermitase DNA" means a DNA sequence coding for a Thermitase variant.

As used herein, "wild-type Thermitase" refers to an enzyme represented by SEQ ID NO:1. The amino acid sequence for Thermitase is further described by Meloun, B., Baudys, M., Kostka, V., Hausdorf, G., Frommel, C., and Hohne, W.E., FEBS LETT., Vol. 183, pp. 195-200 (1985), incorporated herein by reference.

As used herein, the term "Thermitase wild-type amino acid sequence" encompasses SEQ ID NO:1 as well as SEQ ID NO:1 having modifications to the amino acid sequence other than at any of positions 66, 67, 68, 69, 70, 72, 73, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 134, 135, 136, 137, 138, 139, 140, 141, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 191, 192, 193, 194, 195, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223 or 224.

As used herein, "more hydrophilic amino acid" refers to any other amino acid having greater hydrophilicity than a subject amino acid with reference to the hydrophilicity table below. The following hydrophilicity table (Table 1) lists amino acids in descending order of increasing hydrophilicity (see Hopp, T.P., and Woods, K.R., "Prediction of Protein Antigenic Determinants from Amino Acid Sequences", PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCE USA, Vol. 78, pp. 3824-3828, 1981, incorporated herein by reference).

TABLE 1

Amino Acid	Hydrophilicity Value
Trp	-3.4
Phe	-2.5
Tyr	-2.3
Leu, Ile	-1.8
Val	-1.5
Met	-1.3
Cys	-1.0
Ala, His	-0.5
Thr	-0.4
Pro, Gly	-0.0
Gln, Asn	0.2
Ser	0.3
Arg <sup>+</sup> , Lys <sup>+</sup> , Glu <sup>-</sup> , Asp <sup>-</sup>	3.0

Table 1 also indicates which amino acids carry a charge (this characteristic being based on a pH of from about 8-9). The positively charged amino acids are Arg and Lys, the negatively charged amino acids are Glu and Asp, and the remaining amino acids are neutral. In a preferred embodiment of the present invention, the substituting amino acid is either neutral or negatively charged, more preferably negatively charged (i.e., Glu or Asp).

Therefore, for example, the statement "substitute Gln with an equally or more hydrophilic amino acid which is neutral or has a negative charge" means Gln would be substituted with Asn (which is equally hydrophilic to Gln), or Ser, Glu or Asp (which are more hydrophilic than Gln); each of which are neutral or have a negative charge, and have a greater hydrophilicity value as compared to Gln. Likewise, the statement "substitute Pro with a more hydrophilic amino acid which is neutral or has a negative charge" means Pro would be substituted with Gln, Asn, Ser, Glu or Asp.

In one embodiment of the present invention, the Thermitase variant has a modified amino acid sequence of Thermitase wild-type amino acid sequence, wherein the wild-type amino acid sequence comprises a substitution at one or more positions in one or more of the first loop region, the second loop region, the third loop region, the fourth loop region, the fifth loop region or the sixth loop region; whereby the Thermitase variant has



decreased adsorption to, and increased hydrolysis of, an insoluble substrate as compared to the wild-type Thermitase.

In a preferred embodiment of the present invention, the substituting amino acid for one or more of the positions in one or more of the loop regions is, with reference to Table 1, neutral or negatively charged and equally or more hydrophilic, preferably more hydrophilic, than the amino acid at the subject position in the wild-type amino acid sequence.

A. Substitutions in the First Loop Region

When a substitution occurs in the first loop region, the substitution occurs at one or more of positions 66, 67, 68, 69, 70, 72 or 73.

When a substitution occurs at position 66, the substituting amino acid is Asn, Asp, Glu or Ser.

When a substitution occurs at position 67, the substituting amino acid is Asp, Gln, Glu or Ser.

When a substitution occurs at position 68, the substituting amino acid is Asn, Asp, Gln, Glu, Pro or Ser.

When a substitution occurs at position 69, the substituting amino acid is Asp, Gln, Glu or Ser.

When a substitution occurs at position 70, the substituting amino acid is Asn, Asp, Gln, Glu, Pro or Ser.

When a substitution occurs at position 72, the substituting amino acid is Asn, Asp, Gln, Glu, Pro or Ser.

When a substitution occurs at position 73, the substituting amino acid is Asn, Asp, Gln, Glu, Gly, Pro or Ser.

B. Substitutions in the Second Loop Region

When a substitution occurs in the second loop region, the substitution occurs at one or more of positions 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114 or 115.

When a substitution occurs at position 103, the substituting amino acid is Ala, Asn, Asp, Cys, Gln, Glu, Gly, His, Met, Pro, Ser or Thr.

When a substitution occurs at position 104, the substituting amino acid is Ala, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile, Met, Pro, Ser, Thr or Val.

When a substitution occurs at position 105, the substituting amino acid is Glu.

When a substitution occurs at position 106, the substituting amino acid is Asp, Gln, Glu or Ser.

When a substitution occurs at position 107, the substituting amino

acid is Asp or Glu.

When a substitution occurs at position 108, the substituting amino acid is Asn, Asp, Gln, Glu, Pro or Ser.

When a substitution occurs at position 109, the substituting amino acid is Asp or Glu.

When a substitution occurs at position 110, the substituting amino acid is Asn, Asp, Gln, Glu, Pro or Ser.

When a substitution occurs at position 111, the substituting amino acid is Asn, Asp, Gln, Glu, Gly, Pro or Ser.

When a substitution occurs at position 112, the substituting amino acid is Ala, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile, Leu, Met, Phe, Pro, Ser, Thr, Tyr or Val.

When a substitution occurs at position 113, the substituting amino acid is Asn, Asp, Gln, Glu, Gly, Pro or Ser.

When a substitution occurs at position 114, the substituting amino acid is Asn, Asp, Gln, Glu, Gly, His, Pro, Ser or Thr.

When a substitution occurs at position 115, the substituting amino acid is Ala, Asn, Asp, Cys, Gln, Glu, Gly, His, Met, Pro, Ser or Thr.

C. Substitutions in the Third Loop Region

When a substitution occurs in the third loop region, the substitution occurs at one or more of positions 134, 135, 136, 137, 138, 139, 140 or 141, wherein

When a substitution occurs at position 134, the substituting amino acid is Ala, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile, Met, Pro, Ser, Thr or Val.

When a substitution occurs at position 135, the substituting amino acid is Asn, Asp, Gln, Glu, Pro or Ser.

When a substitution occurs at position 136, the substituting amino acid is Asn, Asp, Gln, Glu, Pro or Ser.

When a substitution occurs at position 137, the substituting amino acid is Asn, Asp, Gln, Glu, Gly, Pro or Ser.

When a substitution occurs at position 138, the substituting amino acid is Ala, Asn, Asp, cys, Gln, Glu, Gly, His, Met, Pro, Ser or Thr.

When a substitution occurs at position 139, the substituting amino acid is Asn, Asp, Gln, Glu, Pro or Ser.

When a substitution occurs at position 140, the substituting amino acid is Asp, gln, Glu or Ser. and

When a substitution occurs at position 141, the substituting amino

acid is Asp or Glu.

D. Substitutions in the Fourth Loop Region

When a substitution occurs in the fourth loop region, the substitution occurs at one or more of positions 162, 163, 164, 165, 166, 167, 168, 169, 170 or 171.

When a substitution occurs at position 162, the substituting amino acid is Asn, Asp, Gln, Glu, Pro or Ser.

When a substitution occurs at position 163, the substituting amino acid is Asp, Gln, Glu or Ser.

When a substitution occurs at position 164, the substituting amino acid is Asn, Asp, Gln, Glu, Gly, His, Pro, Ser or Thr.

When a substitution occurs at position 165, the substituting amino acid is Asn, Asp, Gln, Glu, Pro or Ser.

When a substitution occurs at position 166, the substituting amino acid is Asp, Gln, Glu or Ser.

When a substitution occurs at position 167, the substituting amino acid is Asn, Asp, Gln, Glu, Gly, Pro or Ser.

When a substitution occurs at position 168, the substituting amino acid is Asn, Asp, Gln, Glu, Gly, His, Pro, Ser or Thr.

When a substitution occurs at position 169, the substituting amino acid is Asn, Asp, Gln, Glu, Gly or Ser.

When a substitution occurs at position 170, the substituting amino acid is Asp, Gln, Glu or Ser. and

When a substitution occurs at position 171, the substituting amino acid is Ala, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile, Leu, Met, Pro, Ser, Thr or Val.

E. Substitutions in the Fifth Loop Region

When a substitution occurs in the fifth loop region, the substitution occurs at one or more of positions 191, 192, 193, 194 or 195.

When a substitution occurs at position 191, the substituting amino acid is Asp or Glu.

When a substitution occurs at position 192, the substituting amino acid is Asp or Glu.

When a substitution occurs at position 193, the substituting amino acid is Ala, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile, Leu, Met, Pro, Ser, Thr, Tyr or Val.

When a substitution occurs at position 194, the substituting amino

acid is Asp or Glu.

When a substitution occurs at position 195, the substituting amino acid is Asn, Asp, Gln, Glu, Gly, Pro or Ser.

F. Substitutions in the Sixth Loop Region

When a substitution occurs in the sixth loop region, the substitution occurs at one or more of positions 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218 or 219, 220, 221, 223 or 224.

When a substitution occurs at position 204, the substituting amino acid is Asn, Asp, Gln, Glu, Gly, His, Pro, Ser or Thr.

When a substitution occurs at position 205, the substituting amino acid is Asn, Asp, Gln, Glu, Gly or Ser.

When a substitution occurs at position 206, the substituting amino acid is Asn, Asp, Gln, Glu, Pro or Ser.

When a substitution occurs at position 207, the substituting amino acid is Asp or Glu.

When a substitution occurs at position 208, the substituting amino acid is Ala, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile, Leu, Met, Phe, Pro, Ser, Thr, Tyr or Val.

When a substitution occurs at position 209, the substituting amino acid is Ala, Asn, Asp, Cys, Gln, Glu, Gly, His, Leu, Met, Pro, Ser, Thr or Val.

When a substitution occurs at position 210, the substituting amino acid is Ala, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile, Leu, Met, Pro, Ser, Thr or Val.

When a substitution occurs at position 211, the substituting amino acid is Asp or Glu.

When a substitution occurs at position 212, the substituting amino acid is Asn, Asp, Gln, Glu, Gly, Pro or Ser.

When a substitution occurs at position 213, the substituting amino acid is Ala, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile, Leu, Met, Phe, Pro, Ser, Thr or Val.

When a substitution occurs at position 214, the substituting amino acid is Asn, Asp, Gln, Glu, Gly, or Ser.

When a substitution occurs at position 215, the substituting amino acid is Asn, Asp, Gln, Glu, Gly, Pro or Ser.

When a substitution occurs at position 216, the substituting amino acid is Asp or Glu.

When a substitution occurs at position 217, the substituting amino

acid is Asn, Asp, Gln, Glu, Gly, Pro or Ser.

When a substitution occurs at position 218, the substituting amino acid is Ala, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile, Leu, Met, Pro, Ser, Thr or Val.

When a substitution occurs at position 219, the substituting amino acid is Asn, Asp, Gln, Glu, Gly, His, Pro, Ser or Thr.

When a substitution occurs at position 220, the substituting amino acid is Asp or Glu.

When a substitution occurs at position 221, the substituting amino acid is Ala, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile, Met, Pro, Ser, Thr or Val.

When a substitution occurs at position 222, the substituting amino acid is Asp or Glu.

When a substitution occurs at position 223, the substituting amino acid is Asn, Asp, Gln, Glu, Pro or Ser.

When a substitution occurs at position 224, the substituting amino acid is Asn, Asp, Gln, Glu, Gly, Pro or Ser.

#### G. Preparation of enzyme variants

##### Example 1

##### Mutant Thermitase DNA

A phagemid ("TP") containing the wild type Thermitase gene is constructed. The 2.8 Kbp *Pvu* II restriction enzyme fragment of plasmid pUC119, (Vieira, J. and Messing, J., "Production of Single-Stranded Plasmid DNA", 153 METHODS IN ENZYMOLOGY 3-11 (1989)) is cloned into the *Pvu* II site of plasmid pUB110 (Bacillus Genetic Stock Center, Columbus, OH 1E9). The pUC119-pUB110 hybrid plasmid is named pJMA601. Into the *Bam*H1 restriction site of PJMA601 is cloned the polymerase chain reaction-amplified Thermitase gene giving TP. Phagemid TP is transformed into *Escherichia coli* Ung<sup>-</sup> strain CJ236 and a single stranded uracil-containing DNA template is produced using the VCSM13 helper phage (Kunkel, T.A., J.D. Roberts and R.A. Zakour, "Rapid and efficient site-specific mutagenesis without phenotypic selection", METHODS IN ENZYMOLOGY, Vol. 154, pp. 367-382, (1987); as modified by Yuckenberg, P.D., F. Witney, J. Geisselsoder and J. McClary, "Site-directed in vitro mutagenesis using uracil-containing DNA and phagemid vectors", DIRECTED MUTAGENESIS - A PRACTICAL APPROACH, ed. M.J. McPherson, pp. 27-48, (1991); both of which are incorporated herein by reference). A single primer site-directed mutagenesis modification of the method of Zoller and Smith

(Zoller, M.J., and M. Smith, "Oligonucleotide-directed mutagenesis using M13-derived vectors: an efficient and general procedure for the production of point mutations in any fragment of DNA", NUCLEIC ACIDS RESEARCH, Vol. 10, pp. 6487-6500, (1982), incorporated herein by reference) is used to produce all mutants (basically as presented by Yuckenberg, et al., 1991, above). Oligonucleotides are made using an Applied Biosystem Inc. 380B DNA synthesizer. Mutagenesis reaction products are transformed into *Escherichia coli* strain MM294 (American Type Culture Collection *E. coli* 33625). All mutants are confirmed by DNA sequencing and the isolated DNA is transformed into the *Bacillus subtilis* expression strain BG2036 (Yang, M. Y., E. Ferrari and D. J. Henner, (1984), "Cloning of the Neutral Protease Gene of *Bacillus subtilis* and the Use of the Cloned Gene to Create an In Vitro-derived Deletion Mutation", JOURNAL OF BACTERIOLOGY, Vol. 160, pp. 15-21). For some of the mutants a modified TP with a frameshift-stop codon mutation in the corresponding loop is used to produce the uracil template. Oligonucleotides are designed to restore the proper reading frame and to encode for random substitutions at positions 66, 67, 68, 69, 70, 72, 73, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 134, 135, 136, 137, 138, 139, 140, 141, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 191, 192, 193, 194, 195, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223 or 224 (equimolar and/or variable mixtures of all four nucleotides for all three bases at these codons). Mutations that correct for the frameshift-stop and produce a functional enzyme are identified by their ability to digest casein. The random substitutions are determined by DNA sequencing.

#### Example 2

##### Fermentation

The *Bacillus subtilis* cells (BG2036) containing a subtilisin mutant of interest are grown to mid-log phase in a one liter culture of LB-glucose broth and inoculated into a Biostat ED fermenter (B. Braun Biotech, Inc., Allentown, Pennsylvania) in a total volume of 10 liters. The fermentation media contains Yeast Extract, starch, antifoam, buffers and trace minerals (see FERMENTATION: A PRACTICAL APPROACH, Ed. B. McNeil and L. M. Harvey, 1990). The broth is kept at a constant pH of 7.0 during the fermentation run. Chloramphenicol is added for antibiotic selection of mutagenized plasmid. The cells are grown overnight at 37°C to an A<sub>600</sub> of about 60 and harvested.

## Example 3

Purification

The fermentation broth is taken through the following steps to obtain pure enzyme. The broth is cleared of *Bacillus subtilis* cells by centrifugation, and clarified by removing fine particulates with a 100K cutoff membrane. This is followed by concentration on a 10K cutoff membrane, and flow dialysis to reduce the ionic strength and adjust the pH to 5.5 using 0.025M MES buffer (2-(*N*-morpholino)ethanesulfonic acid). The enzyme is further purified by loading it onto either a cation exchange chromatography column or an affinity adsorption chromatography column and eluting it from the column with a NaCl or a propylene glycol gradient (see Scopes, R. K., PROTEIN PURIFICATION PRINCIPLES AND PRACTICE, Springer-Verlag, New York (1984), incorporated herein by reference).

The pNA assay (DeMar, E.G., C. Largman, J.W. Brodrick and M.C. Geokas, ANAL. BIOCHEM., Vol. 99, pp. 316-320, (1979), incorporated herein by reference) is used to determine the active enzyme concentration for fractions collected during gradient elution. This assay measures the rate at which *p*-nitroaniline is released as the enzyme hydrolyzes the soluble synthetic substrate, succinyl-alanine-alanine-proline-phenylalanine-*p*-nitroanilide (sAAPF-*p*NA). The rate of production of yellow color from the hydrolysis reaction is measured at 410 nm on a spectrophotometer and is proportional to the active enzyme concentration. In addition, absorbance measurements at 280 nm are used to determine the total protein concentration. The active enzyme/total-protein ratio gives the enzyme purity, and is used to identify fractions to be pooled for the stock solution.

To avoid autolysis of the enzyme during storage, an equal weight of propylene glycol is added to the pooled fractions obtained from the chromatography column. Upon completion of the purification procedure the purity of the stock enzyme solution is checked with SDS-PAGE (sodium dodecyl sulfate polyacrylamide gel electrophoresis) and the absolute enzyme concentration is determined via an active site titration method using trypsin inhibitor type II-T: turkey egg white purchased from Sigma Chemical Company (St. Louis, Missouri). The measured conversion factors will show which changes made in the enzyme molecule at the various positions result in the enzyme variant having increased activity over the wild-type, against the soluble substrate *p*NA.

In preparation for use, the enzyme stock solution is eluted through a

Sephadex-G25 (Pharmacia, Piscataway, New Jersey) size exclusion column to remove the propylene glycol and exchange the buffer. The MES buffer in the enzyme stock solution is exchanged for 0.1 M Tris buffer (Tris(hydroxymethyl-aminomethane) containing 0.01M  $\text{CaCl}_2$  and pH adjusted to 8.6 with HCl. All experiments are carried out at pH 8.6 in Tris buffer thermostated at 25°C.

#### H. Characterization of enzyme variants

##### Example 4

##### Model Surface Preparation

Aminopropyl controlled pore glass (CPG) purchased from CPG Inc. (Fairfield, New Jersey) is used as a support for covalently attaching the sAAPF-pNA substrate purchased from Bachem, Inc. (Torrence, California). The reaction is carried out in dimethyl sulfoxide and (1-ethyl-3-[3-(dimethylamino)propyl] carbodiimide hydrochloride) (EDC) is used as a coupling agent. Upon completion (monitored by pNA assay), the excess solvent is removed, and the CPG:sAAPF-pNA is rinsed with dimethyl sulfoxide (DMSO) and doubly-distilled water. This is followed by oven drying with a  $\text{N}_2$  purge at about 70°C. The reaction scheme and preparation of the immobilized substrate are conducted as described by Brode, P.F. III, and D.S. Rauch, "Subtilisin BPN': Activity on an Immobilized Substrate," LANGMUIR, Vol. 8, p. 1325-1329, (1992), incorporated herein by reference.

The CPG surface will have  $62,000 \pm 7,000$  pNA molecules/ $\mu\text{m}^2$ . The surface area will remain unchanged from the value of 50.0m<sup>2</sup>/g reported by CPG Inc. for the CPG as received. This suggests that the procedure used to add sAAPF-pNA to CPG does not damage the porous structure (mean diameter is 486 Å).

##### Example 5

##### Surface Hydrolysis Assay

Using CPG:sAAPF-pNA, adsorption of an enzyme variant and hydrolysis of a CPG-bound peptide can be measured in a single experiment. A small volume of enzyme variant stock solution is added to a flask containing Tris buffer and CPG:sAAPF-pNA which has been degassed. The flask is shaken on a wrist-action shaker for a period of 90 minutes during which the shaker is stopped at various time intervals (for example, every 2 minutes during the early stages of adsorption hydrolysis - e.g., the first 20 minutes - and every 10 minutes towards the end of the



experiment). The CPG:sAAPF-pNA is allowed to settle and the solution is sampled. Both the experimental procedure and the calculation of the adsorption and hydrolysis are conducted as described by Brode *et al.*, 1992, above.

All enzymes are monitored for stability against autolysis and should show no appreciable autolytic loss over the time course of this experiment. Therefore, enzyme adsorption can be determined by measuring solution depletion. The difference between the initial enzyme variant concentration and the concentration measured at each individual time point gives the amount of enzyme variant adsorbed. The amount of pNA hydrolyzed from the surface is measured by taking an absorbance reading on an aliquot of the sample at 410 nm. The total amount of pNA hydrolyzed is calculated by adding the amount sampled and the amount remaining in the flask. This value is corrected by subtracting the amount of pNA that is hydrolyzed by Tris buffer at pH 8.6 when no enzyme is present. This base-hydrolysis ranges from 7-29% of the total hydrolysis depending on the efficiency of the enzyme.

#### Example 6

##### Soluble Substrate Kinetic Analysis

The rates of hydrolysis of the soluble substrate sAAPF-pNA are monitored by measuring the adsorbance increase as a function of time at 410 nm on a DU-70 spectrophotometer. The enzyme concentration is held constant and is prepared to be in the range of 6-10 nanomolar while the substrate concentration is varied from 90-700  $\mu$ M sAAPF-pNA for each kinetic determination. An adsorbance data point is taken each second over a period of 900 seconds and the data are transferred to a LOTUS™ spreadsheet (Lotus Development Corporation, Cambridge, Massachusetts). Analysis for kinetic parameters is conducted by the standard Lineweaver Burk analysis in which the data in the initial part of the run (generally the first minute) are fit to a linear regression curve to give  $v_0$ . The  $v_0$  and  $s_0$  data are plotted in the standard inverse fashion to give  $K_M$  and  $k_{cat}$ .

I. Example Thermitase variants

Thermitase variants of the present invention which have decreased adsorption to and increased hydrolysis of surface bound substrates are exemplified in Tables 2-36, below. In describing the specific mutations, the original amino acid occurring in wild-type is given first, the position number second, and the substituted amino acid third.

TABLE 2

Loop 1 - Single Mutation Variants
Gln66Asn
Gln66Asp
Gln66Glu
Gln66Ser
Asn67Asp
Asn67Gln
Asn67Glu
Asn67Ser
Gly68Asn
Gly68Asp
Gly68Gln
Gly68Glu
Gly68Pro
Gly68Ser
Asn69Asp
Asn69Gln
Asn69Glu
Asn69Ser
Gly70Asn
Gly70Asp
Gly70Gln
Gly70Glu
Gly70Pro
Gly70Ser
Gly72Asn
Gly72Asp
Gly72Gln
Gly72Glu
Gly72Pro
Gly72Ser
Thr73Asn
Thr73Asp
Thr73Gln
Thr73Glu
Thr73Gly
Thr73Pro
Thr73Ser

TABLE 3

Loop 1 - Double Mutation Variants
Asn68Asp + Gly72Pro
Gly69Asn + Thr73Pro
Asn66Glu + Gly69Ser
Asn66Glu + Gly67Ser
Gly69Gln + Thr73Pro
Gly67Ser + Gly72Ser
Gly67Asp + Thr73Gly
Gly69Pro + Ser70Asp
Gly67Pro + Gly69Pro
Asn66Glu + Thr73Gly
Gly69Gln + Gly72Asp
Asn66Gln + Thr73Gly
Asn68Asp + Thr73Ser
Asn66Gln + Gly69Asn
Gly67Glu + Thr73Ser
Gly72Glu + Thr73Pro
Asn66Glu + Thr73Pro
Gly72Asp + Thr73Gln
Asn68Ser + Gly69Glu
Gly67Pro + Gly69Ser
Gly67Pro + Gly72Asp
Gly69Ser + Gly72Glu
Ser70Asp + Thr73Gln
Gly67Ser + Asn68Glu
Gly69Ser + Thr73Ser
Asn68Asp + Gly69Ser
Asn66Ser + Gly72Asp
Gly69Asp + Gly72Ser
Asn68Ser + Thr73Pro
Ser70Asp + Thr73Ser
Ser70Glu + Gly72Asn
Gly69Asp + Thr73Pro
Gly67Glu + Thr73Gly
Gly72Asp + Thr73Ser
Ser70Asp + Thr73Asn
Asn66Ser + Gly69Glu
Gly72Ser + Thr73Gln
Ser70Glu + Thr73Ser
Asn66Gln + Gly69Ser

TABLE 4

Loop 1 - Triple Mutation Variants
Ser70Asp + Gly72Gln + Thr73Gly
Gly67Ser + Asn68Gln + Ser70Asp
Asn66Ser + Gly67Asn + Thr73Gln
Asn66Gln + Ser70Asp + Gly72Asn
Ser70Glu + Gly72Gln + Thr73Ser

Gly67Pro + Asn68Glu + Thr73Ser  
 Asn66Ser + Gly69Gln + Gly72Gln  
 Asn66Ser + Ser70Asp + Thr73Gln  
 Gly67Gln + Gly69Asp + Thr73Gln  
 Gly69Pro + Gly72Glu + Thr73Ser  
 Gly67Gln + Gly69Asn + Thr73Asn  
 Asn66Ser + Gly69Asp + Thr73Asn  
 Gly69Asp + Gly72Ser + Thr73Ser  
 Asn68Asp + Gly72Gln + Thr73Ser  
 Asn66Gln + Asn68Ser + Thr73Asn  
 Asn68Ser + Gly69Ser + Ser70Asp  
 Asn68Gln + Gly69Ser + Thr73Gln  
 Gly67Ser + Gly69Pro + Gly72Glu  
 Gly67Asn + Gly69Ser + Thr73Asn  
 Gly67Gln + Gly72Asn + Thr73Gln  
 Gly67Pro + Asn68Gln + Gly69Pro  
 Gly69Asn + Ser70Glu + Thr73Ser  
 Asn66Ser + Ser70Glu + Gly72Asn  
 Gly69Gln + Ser70Asp + Gly72Asn  
 Gly67Ser + Ser70Glu + Thr73Gln  
 Asn66Glu + Gly67Gln + Thr73Pro  
 Gly67Ser + Gly72Glu + Thr73Ser  
 Gly67Pro + Gly69Gln + Gly72Asp  
 Asn66Ser + Gly67Glu + Thr73Pro  
 Asn66Ser + Gly67Glu + Asn68Ser  
 Gly67Asn + Asn68Glu + Gly69Asp  
 Asn68Glu + Gly69Glu + Gly72Pro  
 Asn68Glu + Gly69Glu + Thr73Asn  
 Gly67Glu + Asn68Asp + Gly69Gln  
 Gly67Glu + Asn68Asp + Thr73Gln  
 Asn66Asp + Gly67Asp + Gly72Ser  
 Asn66Glu + Gly67Glu + Thr73Pro

TABLE 5

---

 Loop 1 - Quadruple Mutation Variants
 

---

Asn66Gln + Gly67Ser + Gly69Asp + Gly72Pro  
 Asn68Ser + Ser70Glu + Gly72Gln + Thr73Pro  
 Asn66Gln + Gly67Gln + Ser70Asp + Thr73Asn  
 Gly67Ser + Gly69Asp + Gly72Gln + Thr73Gly  
 Asn66Glu + Gly67Ser + Asn68Ser + Thr73Asn  
 Gly67Ser + Asn68Asp + Gly72Asn + Thr73Gly  
 Asn66Ser + Gly67Glu + Gly69Gln + Thr73Gly  
 Asn68Glu + Gly69Asp + Gly72Asn + Thr73Asn  
 Gly67Glu + Asn68Glu + Gly72Asn + Thr73Asn  
 Asn66Glu + Gly67Glu + Asn68Gln + Gly72Pro  
 Asn66Glu + Gly67Glu + Asn68Ser + Gly72Asn  
 Asn66Glu + Gly67Glu + Gly72Pro + Thr73Asn  
 Gly67Ser + Gly69Asp + Ser70Glu + Thr73Gln  
 Asn66Ser + Gly69Glu + Ser70Glu + Thr73Gly  
 Gly67Pro + Asn68Glu + Gly69Glu + Ser70Glu  
 Gly67Ser + Asn68Glu + Gly69Asp + Ser70Asp

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Gly67Asn + Asn68Asp + Gly69Asp + Ser70Glu  
 Gly67Asp + Asn68Gln + Gly69Glu + Ser70Asp  
 Asn66Gln + Gly67Asp + Gly69Asp + Ser70Asp  
 Gly67Asp + Asn68Asp + Ser70Asp + Thr73Asn  
 Gly67Asp + Asn68Asp + Gly69Pro + Ser70Glu  
 Gly67Asp + Asn68Gln + Ser70Glu + Gly72Pro  
 Asn66Gln + Gly67Glu + Ser70Asp + Gly72Gln  
 Gly67Glu + Ser70Glu + Gly72Gln + Thr73Ser  
 Gly67Asp + Asn68Gln + Ser70Asp + Gly72Ser  
 Gly67Glu + Ser70Asp + Gly72Ser + Thr73Ser

TABLE 6

---

 Loop 2 - Single Mutation Variants
 

---

Val103Ala  
 Val103Asn  
 Val103Asp  
 Val103Cys  
 Val103Gln  
 Val103Glu  
 Val103Gly  
 Val103His  
 Val103Met  
 Val103Pro  
 Val103Ser  
 Val103Thr  
 Leu104Ala  
 Leu104Asn  
 Leu104Asp  
 Leu104Cys  
 Leu104Gln  
 Leu104Glu  
 Leu104Gly  
 Leu104His  
 Leu104Ile  
 Leu104Met  
 Leu104Pro  
 Leu104Ser  
 Leu104Thr  
 Leu104Val  
 Asp105Glu  
 Asn106Asp  
 Asn106Gln  
 Asn106Glu  
 Asn106Ser  
 Ser107Asp  
 Ser107Glu  
 Gly108Asn  
 Gly108Asp  
 Gly108Gln  
 Gly108Glu  
 Gly108Pro

Gly108Ser  
Ser109Asp  
Ser109Glu  
Gly110Asn  
Gly110Asp  
Gly110Gln  
Gly110Glu  
Gly110Pro  
Gly110Ser  
Thr111Asn  
Thr111Asp  
Thr111Gln  
Thr111Glu  
Thr111Gly  
Thr111Pro  
Thr111Ser  
Trp112Ala  
Trp112Asn  
Trp112Asp  
Trp112Cys  
Trp112Gln  
Trp112Glu  
Trp112Gly  
Trp112His  
Trp112Ile  
Trp112Leu  
Trp112Met  
Trp112Phe  
Trp112Pro  
Trp112Ser  
Trp112Thr  
Trp112Tyr  
Trp112Val  
Thr113Asn  
Thr113Asp  
Thr113Gln  
Thr113Glu  
Thr113Gly  
Thr113Pro  
Thr113Ser  
Ala114Asn  
Ala114Asp  
Ala114Gln  
Ala114Glu  
Ala114Gly  
Ala114His  
Ala114Pro  
Ala114Ser  
Ala114Thr  
Val115Ala  
Val115Asn  
Val115Asp  
Val115Cys

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Val115Gln  
 Val115Glu  
 Val115Gly  
 Val115His  
 Val115Met  
 Val115Pro  
 Val115Ser  
 Val115Thr

TABLE 7

---

 Loop 2 - Double Mutation Variants
 

---

Val103Gln + Ser109Glu  
 Asp105Glu + Gly108Gln  
 Asp105Glu + Gly110Asn  
 Asp105Glu + Gly110Pro  
 Val103Asp + Thr111Gln  
 Leu104Gln + Gly108Asp  
 Leu104Gly + Ser107Glu  
 Thr111Pro + Ala114Ser  
 Asn106Gln + Ser109Asp  
 Asp105Glu + Gly108Pro  
 Thr113Asp + Val115Gly  
 Val103Met + Leu104Gln  
 Gly108Gln + Thr113Asn  
 Gly110Glu + Ala114Gly  
 Thr111Gln + Val115Gln  
 Gly110Glu + Thr111Gln  
 Gly108Pro + Ala114Glu  
 Gly108Ser + Val115Gly  
 Asp105Glu + Thr113Gln  
 Leu104His + Ser107Asp  
 Asp105Glu + Ala114Gln  
 Gly110Gln + Thr113Ser  
 Val103His + Ala114Gln  
 Asp105Glu + Trp112Ile  
 Thr111Ser + Thr113Asp  
 Leu104His + Thr111Glu  
 Asn106Gln + Trp112Tyr  
 Leu104Gly + Thr113Gln  
 Val103Thr + Val115Asn  
 Val103Thr + Asn106Ser  
 Val103Pro + Thr111Pro  
 Ser109Glu + Val115Met  
 Val103Ala + Asn106Gln  
 Val103Cys + Thr111Gln  
 Trp112Gly + Thr113Gly  
 Val103Gly + Asp105Glu  
 Gly110Asn + Thr113Gly  
 Thr111Asn + Thr113Glu  
 Val103Glu + Trp112Met  
 Leu104Pro + Ser109Asp

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Gly108Gln + Ala114Gly  
 Leu104Met + Val115Glu  
 Leu104Gly + Ala114Ser  
 Thr113Ser + Val115Ala  
 Asp105Glu + Asn106Gln  
 Val103Thr + Asp105Glu  
 Gly110Glu + Val115Met  
 Val103Gly + Gly110Glu  
 Ser109Asp + Gly110Gln  
 Thr111Asp + Ala114Ser  
 Asn106Glu + Ala114Pro  
 Ser109Glu + Thr113Gly  
 Gly108Asn + Trp112Glu  
 Val103Pro + Asn106Glu  
 Asp105Glu + Trp112Leu  
 Leu104Cys + Asp105Glu  
 Leu104Cys + Thr111Ser  
 Leu104Thr + Ser109Asp  
 Val103Asn + Val115Glu  
 Val103Ser + Gly108Glu

TABLE 8

## Loop 2 - Triple Mutation Variants

Val103Gln + Leu104Thr + Ser109Glu  
 Leu104Ser + Asp105Glu + Trp112Phe  
 Asn106Gln + Gly108Ser + Gly110Gln  
 Ser109Asp + Thr111Asn + Trp112Thr  
 Asn106Ser + Gly108Gln + Ser109Asp  
 Asp105Glu + Gly108Pro + Val115Gly  
 Asn106Ser + Ser107Asp + Val115Gly  
 Asn106Ser + Trp112Gln + Val115Glu  
 Leu104Pro + Ser109Asp + Thr113Gly  
 Leu104Asp + Gly110Gln + Val115Pro  
 Gly108Asn + Thr111Ser + Ala114Pro  
 Leu104Ser + Asn106Ser + Thr111Glu  
 Val103His + Leu104Gln + Asn106Asp  
 Asn106Gln + Trp112Asn + Ala114Asp  
 Val103Met + Gly110Pro + Thr111Pro  
 Leu104Thr + Gly108Glu + Gly110Asn  
 Leu104Ala + Ser109Asp + Trp112Cys  
 Gly108Glu + Gly110Pro + Val115Gly  
 Leu104Pro + Thr113Asn + Ala114Asp  
 Val103Asn + Leu104Met + Asn106Glu  
 Val103Cys + Ser109Glu + Val115Cys  
 Val103Gly + Leu104Val + Ser107Asp  
 Gly108Pro + Ser109Glu + Trp112Gln  
 Leu104Met + Trp112Cys + Thr113Asn  
 Asn106Asp + Trp112Ile + Ala114Ser  
 Val103Gly + Ser109Glu + Ala114Pro  
 Leu104Ile + Trp112Leu + Thr113Gly  
 Asn106Gln + Gly108Asn + Ser109Glu



Val103Asn + Trp112Pro + Ala114Asp  
 Gly108Asn + Gly110Ser + Trp112Gly  
 Gly110Asp + Trp112Leu + Val115His  
 Val103Gly + Leu104Gly + Gly108Asp  
 Leu104Ala + Thr111Ser + Val115Ala  
 Thr111Ser + Thr113Asn + Val115His  
 Asp105Glu + Gly108Gln + Val115Gly  
 Leu104Gly + Ala114Ser + Val115Ala  
 Val103His + Leu104Gly + Asn106Glu  
 Val103Ser + Gly110Glu + Thr113Pro  
 Val103Pro + Gly110Asn + Ala114Glu  
 Leu104Met + Gly110Asp + Val115Pro  
 Leu104Asp + Thr111Gln + Trp112Ala  
 Ser109Asp + Gly110Pro + Trp112Asn  
 Leu104Asp + Asp105Glu + Trp112Cys  
 Gly108Glu + Ser109Glu + Ala114Thr  
 Gly108Asp + Ser109Asp + Thr111Pro  
 Gly108Glu + Ser109Glu + Ala114Asn  
 Asn106Ser + Ser109Glu + Gly110Asp  
 Asn106Gln + Ser109Asp + Gly110Asp  
 Val103Asp + Leu104Asp + Gly108Pro  
 Leu104Thr + Asp105Glu + Asn106Glu  
 Asp105Glu + Asn106Asp + Thr113Asn  
 Gly108Gln + Thr113Glu + Ala114Glu  
 Leu104Ser + Thr111Glu + Trp112Glu  
 Asn106Asp + Ser107Asp + Gly108Asp  
 Asp105Glu + Ser109Asp + Gly110Glu  
 Trp112Ala + Thr113Asp + Val115Asp  
 Val103Gln + Asp105Glu + Gly108Asp  
 Leu104Gln + Asp105Glu + Ser107Glu  
 Ser107Asp + Ser109Asp + Thr111Gly  
 Val103Cys + Ser107Asp + Ser109Glu

TABLE 9

---

 Loop 2 - Quadruple Mutation Variants
 

---

Asp105Glu + Gly110Ser + Trp112Phe + Thr113Ser  
 Val103Ala + Ser107Asp + Gly110Pro + Ala114Asn  
 Val103Gln + Gly108Pro + Thr111Ser + Ala114Glu  
 Val103Thr + Ser107Asp + Thr113Gln + Val115Cys  
 Leu104Asn + Gly110Ser + Thr113Glu + Val115Gln  
 Val103Pro + Gly108Glu + Trp112His + Val115Met  
 Val103Ala + Asn106Ser + Gly108Asp + Thr111Asn  
 Val103Ala + Leu104Asp + Gly110Ser + Val115Ala  
 Ser109Glu + Trp112Pro + Ala114Asn + Val115Met  
 Val103Thr + Asn106Asp + Gly108Ser + Gly110Gln  
 Val103Pro + Leu104Pro + Asn106Ser + Gly110Asn  
 Ser109Glu + Gly110Pro + Thr111Ser + Thr113Ser  
 Asn106Gln + Gly108Ser + Trp112Tyr + Ala114Thr  
 Leu104Cys + Gly110Gln + Thr111Asp + Thr113Gly  
 Leu104Ser + Asp105Glu + Gly108Gln + Val115Asn  
 Val103Asp + Gly108Asn + Thr113Pro + Ala114Pro

Val103Asp + Leu104Asn + Thr113Asn + Val115Ala  
 Val103Asn + Gly110Asp + Thr111Asn + Val115Cys  
 Val103Ser + Asn106Glu + Gly110Gln + Val115Met  
 Leu104Val + Thr111Gln + Trp112His + Ala114Ser  
 Leu104Ser + Asp105Glu + Gly110Gln + Thr111Asn  
 Val103Ala + Asp105Glu + Asn106Ser + Gly110Asn  
 Val103Glu + Gly110Ser + Trp112Phe + Val115Gly  
 Asn106Glu + Thr113Asn + Ala114Pro + Val115Asn  
 Leu104Glu + Asp105Glu + Gly108Ser + Gly110Asn  
 Gly108Glu + Ser109Glu + Thr111Asn + Ala114Pro  
 Gly108Glu + Ser109Glu + Thr111Gln + Ala114Thr  
 Leu104Ile + Gly108Pro + Ser109Glu + Gly110Asp  
 Val103Glu + Leu104Asp + Trp112Cys + Thr113Ser  
 Asp105Glu + Asn106Glu + Gly110Pro + Thr113Gly  
 Gly110Gln + Thr111Glu + Trp112Glu + Thr113Gly  
 Ser107Asp + Gly108Asp + Ser109Asp + Trp112Val  
 Asn106Asp + Ser107Asp + Gly108Glu + Val115Pro  
 Asp105Glu + Ser107Glu + Gly108Asp + Gly110Ser  
 Asp105Glu + Gly108Asp + Ser109Glu + Thr111Gln  
 Asp105Glu + Gly108Glu + Ser109Glu + Thr111Pro  
 Leu104Asp + Ser109Glu + Trp112Asn + Ala114Gly  
 Val103Ser + Leu104Glu + Gly108Asn + Ser109Glu  
 Asp105Glu + Ser109Glu + Gly110Glu + Trp112Gln  
 Val103Ser + Asp105Glu + Gly108Glu + Val115Pro  
 Asp105Glu + Ser107Asp + Trp112Leu + Thr113Gly  
 Leu104His + Asp105Glu + Asn106Ser + Ser107Asp  
 Asp105Glu + Ser107Glu + Ser109Asp + Val115Gln  
 Asp105Glu + Ser107Glu + Ser109Glu + Thr113Gly  
 Ser107Glu + Ser109Glu + Thr111Gln + Thr113Gln  
 Ser107Glu + Ser109Glu + Trp112Val + Val115Pro  
 Ser107Glu + Ser109Glu + Trp112Ile + Val115Gln  
 Asp105Glu + Ser109Glu + Gly110Gln + Val115Pro  
 Leu104Glu + Asp105Glu + Ser107Glu + Ala114Gly  
 Asp105Glu + Asn106Glu + Gly108Pro + Ser109Asp  
 Leu104Asn + Asp105Glu + Gly110Asp + Ala114Gly  
 Ser107Glu + Gly108Gln + Ser109Asp + Gly110Asp  
 Gly108Gln + Gly110Glu + Trp112Asp + Val115Glu  
 Val103Asp + Leu104Ser + Asp105Glu + Val115Gln  
 Leu104Glu + Asn106Glu + Gly110Glu + Val115Asn  
 Leu104Asp + Gly110Glu + Thr111Ser + Trp112Asp  
 Ser109Asp + Gly110Glu + Thr113Asn + Val115Glu  
 Val103Ser + Gly108Asp + Gly110Glu + Thr111Glu  
 Leu104Gly + Asn106Glu + Gly108Asn + Ser109Glu  
 Val103Asp + Leu104Gly + Thr111Glu + Val115Met

TABLE 10

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 Loop 3 - Single Mutation Variants
 

---

Leu134Ala  
 Leu134Asn  
 Leu134Asp  
 Leu134Cys

Leu134Gln  
Leu134Glu  
Leu134Gly  
Leu134His  
Leu134Ile  
Leu134Met  
Leu134Pro  
Leu134Ser  
Leu134Thr  
Leu134Val  
Gly135Asn  
Gly135Asp  
Gly135Gln  
Gly135Glu  
Gly135Pro  
Gly135Ser  
Gly136Asn  
Gly136Asp  
Gly136Gln  
Gly136Glu  
Gly136Pro  
Gly136Ser  
Thr137Asn  
Thr137Asp  
Thr137Gln  
Thr137Glu  
Thr137Gly  
Thr137Pro  
Thr137Ser  
Val138Ala  
Val138Asn  
Val138Asp  
Val138Cys  
Val138Gln  
Val138Glu  
Val138Gly  
Val138His  
Val138Met  
Val138Pro  
Val138Ser  
Val138Thr  
Gly139Asn  
Gly139Asp  
Gly139Gln  
Gly139Glu  
Gly139Pro  
Gly139Ser  
Asn140Asp  
Asn140Gln  
Asn140Glu  
Asn140Ser  
Ser141Asp  
Ser141Glu

---

TABLE 11

Loop 3 - Double Mutation Variants	
Leu134Val + Ser141Asp	
Leu134Val + Thr137Glu	
Gly135Asn + Ser141Asp	
Gly135Ser + Thr137Ser	
Leu134Met + Gly139Asp	
Leu134Thr + Gly139Gln	
Gly139Gln + Asn140Glu	
Gly139Pro + Ser141Asp	
Leu134Glu + Val138Asn	
Leu134Asn + Val138Ala	
Leu134Ser + Asn140Glu	
Val138Asn + Ser141Asp	
Gly135Glu + Val138Ser	
Leu134Ile + Gly139Glu	
Leu134Ile + Gly135Asn	
Leu134Met + Asn140Asp	
Gly136Ser + Gly139Asp	
Gly136Pro + Val138Asn	
Leu134Gly + Gly135Pro	
Leu134Gly + Ser141Glu	
Gly135Pro + Gly139Pro	
Leu134Thr + Gly139Pro	
Gly135Glu + Asn140Ser	
Thr137Gln + Gly139Asp	
Gly135Asp + Asn140Ser	
Leu134Ser + Asn140Asp	
Gly136Gln + Gly139Asp	
Leu134Pro + Ser141Glu	
Leu134His + Asn140Gln	
Leu134Pro + Gly135Glu	
Gly135Pro + Gly136Asp	
Val138Thr + Ser141Asp	
Val138Pro + Ser141Asp	
Leu134Gln + Thr137Glu	
Val138Ser + Ser141Asp	
Val138Thr + Asn140Gln	
Leu134Glu + Val138Gln	
Gly139Asn + Ser141Glu	
Gly139Pro + Ser141Glu	
Gly135Gln + Gly139Glu	
Thr137Pro + Val138Gly	
Gly135Asp + Gly136Gln	
Gly135Asn + Val138Thr	
Thr137Asn + Ser141Glu	
Gly135Pro + Val138Glu	
Gly135Glu + Thr137Ser	
Gly135Asp + Gly139Pro	
Leu134Ala + Gly135Gln	

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Val138Gln + Ser141Asp  
 Gly135Ser + Gly139Asp  
 Thr137Ser + Val138Met  
 Leu134Ala + Gly136Gln  
 Leu134Glu + Asn140Gln  
 Gly136Asn + Asn140Gln  
 Gly136Gln + Ser141Asp  
 Val138Ser + Ser141Glu  
 Leu134Thr + Thr137Glu  
 Gly135Ser + Gly139Pro  
 Val138Cys + Gly139Asp  
 Thr137Gly + Gly139Asp

TABLE 12

## Loop 3 - Triple Mutation Variants

Gly135Ser + Val138Cys + Ser141Asp  
 Gly135Gln + Thr137Glu + Val138Cys  
 Leu134Ser + Gly135Pro + Gly136Glu  
 Leu134Pro + Val138Cys + Asn140Ser  
 Leu134Val + Gly135Asp + Val138Met  
 Leu134Pro + Gly135Pro + Gly139Glu  
 Gly135Gln + Val138Asp + Gly139Ser  
 Gly136Pro + Val138His + Ser141Asp  
 Leu134Val + Gly136Ser + Asn140Asp  
 Thr137Asp + Val138Met + Gly139Gln  
 Leu134Val + Gly136Pro + Thr137Gly  
 Gly135Gln + Val138Gln + Asn140Asp  
 Leu134His + Gly136Glu + Asn140Gln  
 Gly135Pro + Gly139Gln + Ser141Asp  
 Gly136Asn + Val138Asn + Ser141Asp  
 Gly135Asp + Val138Thr + Gly139Gln  
 Gly136Pro + Thr137Asp + Gly139Ser  
 Leu134Gly + Gly136Gln + Gly139Ser  
 Leu134Val + Gly135Glu + Thr137Gly  
 Leu134Thr + Val138Gln + Gly139Ser  
 Gly136Asp + Thr137Asn + Val138Gly  
 Gly135Asp + Val138Ser + Asn140Gln  
 Gly136Asn + Val138Glu + Asn140Gln  
 Leu134Cys + Thr137Glu + Val138Gly  
 Leu134Asn + Gly135Glu + Gly139Ser  
 Gly135Ser + Val138His + Asn140Glu  
 Leu134Ala + Gly139Ser + Asn140Gln  
 Gly135Asp + Thr137Pro + Val138Cys  
 Leu134Cys + Val138Gly + Asn140Asp  
 Leu134Thr + Val138Ser + Gly139Asp  
 Leu134Cys + Thr137Gln + Ser141Glu  
 Leu134Val + Gly136Pro + Gly139Glu  
 Leu134Ala + Gly135Asn + Ser141Glu  
 Gly136Gln + Thr137Gln + Val138Pro  
 Leu134Asp + Gly135Pro + Gly139Ser  
 Gly135Ser + Val138Gly + Ser141Asp

Leu134Val + Thr137Asp + Gly139Gln  
 Leu134His + Gly135Pro + Gly139Gln  
 Thr137Pro + Val138Gln + Asn140Asp  
 Thr137Gln + Val138Cys + Gly139Glu  
 Thr137Ser + Gly139Glu + Asn140Gln  
 Gly135Gln + Thr137Gln + Asn140Asp  
 Gly135Gln + Thr137Gln + Val138Asp  
 Leu134His + Gly139Gln + Asn140Asp  
 Leu134Ala + Thr137Asn + Gly139Glu  
 Val138Pro + Gly139Pro + Asn140Gln  
 Gly135Asn + Asn140Ser + Ser141Glu  
 Leu134Ile + Gly135Asp + Val138Cys  
 Thr137Gln + Gly139Ser + Asn140Ser  
 Leu134Ala + Gly136Pro + Asn140Asp  
 Gly135Ser + Gly139Asp + Asn140Ser  
 Leu134Thr + Gly135Asn + Ser141Glu  
 Leu134Gly + Thr137Ser + Val138Ala  
 Leu134Thr + Gly135Gln + Val138Pro  
 Gly135Gln + Gly136Asp + Thr137Ser  
 Gly135Ser + Val138Thr + Asn140Asp  
 Leu134Cys + Thr137Ser + Val138Glu  
 Gly136Ser + Thr137Asn + Ser141Asp  
 Leu134Thr + Gly135Glu + Gly139Gln  
 Leu134Thr + Gly135Asp + Thr137Ser

TABLE 13

## Loop 3 - Quadruple Mutation Variants

Leu134Met + Thr137Pro + Asn140Ser + Ser141Glu  
 Leu134Asn + Gly136Pro + Thr137Ser + Val138Gln  
 Leu134Gln + Gly136Gln + Val138Ser + Gly139Ser  
 Gly135Glu + Gly136Asn + Thr137Pro + Asn140Gln  
 Leu134Ser + Gly136Asn + Gly139Gln + Ser141Glu  
 Leu134Gln + Val138Pro + Gly139Ser + Ser141Asp  
 Leu134Pro + Gly135Asn + Thr137Glu + Asn140Gln  
 Gly135Asp + Thr137Gly + Val138Gln + Gly139Ser  
 Leu134Val + Gly135Asp + Thr137Asn + Asn140Ser  
 Leu134Thr + Gly135Glu + Gly136Ser + Thr137Ser  
 Gly136Pro + Thr137Gln + Gly139Gln + Asn140Glu  
 Leu134His + Gly135Glu + Val138Asn + Asn140Gln  
 Leu134Gln + Gly135Ser + Thr137Asn + Ser141Glu  
 Gly135Gln + Gly139Asn + Asn140Gln + Ser141Asp  
 Thr137Gln + Val138His + Gly139Asn + Ser141Glu  
 Leu134Thr + Gly135Gln + Val138Thr + Asn140Asp  
 Leu134Met + Gly135Glu + Thr137Ser + Val138Ser  
 Val138Thr + Gly139Ser + Asn140Ser + Ser141Glu  
 Leu134Met + Gly136Pro + Val138Cys + Gly139Pro  
 Leu134His + Gly136Pro + Thr137Gln + Gly139Glu  
 Gly135Asn + Gly136Gln + Val138Cys + Ser141Glu  
 Leu134Asn + Thr137Ser + Val138Gln + Ser141Asp  
 Gly135Gln + Gly136Pro + Thr137Asn + Asn140Ser  
 Gly135Asn + Gly139Ser + Asn140Gln + Ser141Asp

Leu134Met + Gly136Pro + Val138Met + Ser141Glu  
 Leu134Cys + Thr137Gln + Val138Pro + Ser141Asp  
 Gly136Asn + Thr137Gln + Gly139Asn + Asn140Ser  
 Leu134Asn + Thr137Ser + Gly139Ser + Asn140Asp  
 Gly135Asp + Gly136Asp + Val138Met + Asn140Ser  
 Gly135Glu + Gly136Asp + Val138His + Gly139Gln  
 Leu134Ser + Gly136Glu + Thr137Glu + Val138Thr  
 Leu134Thr + Val138Gln + Asn140Glu + Ser141Glu  
 Gly135Gln + Thr137Asn + Asn140Glu + Ser141Glu  
 Gly135Gln + Thr137Gly + Val138Asp + Gly139Asp  
 Leu134Thr + Gly135Glu + Gly136Asp + Thr137Asp  
 Val138Asn + Gly139Asp + Asn140Glu + Ser141Asp  
 Leu134Ile + Gly139Glu + Asn140Asp + Ser141Asp  
 Gly136Pro + Gly139Glu + Asn140Glu + Ser141Asp  
 Leu134Gln + Gly139Asp + Asn140Asp + Ser141Glu  
 Leu134Val + Gly136Glu + Val138Asp + Asn140Gln  
 Gly136Asp + Thr137Gln + Val138Asp + Gly139Asn  
 Gly136Glu + Thr137Glu + Val138His + Gly139Asp  
 Leu134His + Thr137Gln + Val138Glu + Asn140Glu  
 Leu134Gly + Gly135Ser + Thr137Glu + Gly139Glu  
 Leu134Gln + Gly135Pro + Thr137Asp + Gly139Asp  
 Leu134His + Gly135Asn + Thr137Asp + Gly139Glu  
 Leu134Val + Gly135Glu + Thr137Asp + Gly139Gln  
 Gly135Asn + Thr137Ser + Gly139Asp + Ser141Asp  
 Leu134Cys + Thr137Asn + Gly139Glu + Ser141Asp  
 Leu134Ile + Thr137Pro + Gly139Asp + Ser141Asp  
 Leu134Thr + Val138Asn + Gly139Glu + Ser141Asp  
 Gly135Pro + Thr137Gly + Gly139Glu + Ser141Asp  
 Gly136Ser + Val138Ala + Gly139Glu + Ser141Glu  
 Leu134Thr + Gly139Asp + Asn140Ser + Ser141Asp  
 Gly135Ser + Thr137Gly + Gly139Glu + Ser141Asp  
 Leu134Met + Gly135Asn + Gly139Asp + Ser141Glu  
 Thr137Pro + Val138Gly + Gly139Asp + Ser141Asp  
 Gly136Glu + Thr137Pro + Val138Gln + Gly139Asp  
 Thr137Asn + Val138Glu + Gly139Asp + Ser141Asp  
 Leu134Thr + Val138Glu + Gly139Glu + Ser141Glu

TABLE 14

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 Loop 4 - Single Mutation Variants
 

---

Gly162Asn  
 Gly162Asp  
 Gly162Gln  
 Gly162Glu  
 Gly162Pro  
 Gly162Ser  
 Asn163Asp  
 Asn163Gln  
 Asn163Glu  
 Asn163Ser  
 Ala164Asn  
 Ala164Asp

Ala164Gln  
Ala164Glu  
Ala164Gly  
Ala164His  
Ala164Pro  
Ala164Ser  
Ala164Thr  
Gly165Asn  
Gly165Asp  
Gly165Gln  
Gly165Glu  
Gly165Pro  
Gly165Ser  
Asn166Asp  
Asn166Gln  
Asn166Glu  
Asn166Ser  
Thr167Asn  
Thr167Asp  
Thr167Gln  
Thr167Glu  
Thr167Gly  
Thr167Pro  
Thr167Ser  
Ala168Asn  
Ala168Asp  
Ala168Gln  
Ala168Glu  
Ala168Gly  
Ala168His  
Ala168Pro  
Ala168Ser  
Ala168Thr  
Pro169Asn  
Pro169Asp  
Pro169Gln  
Pro169Glu  
Pro169Gly  
Pro169Ser  
Asn170Asp  
Asn170Gln  
Asn170Glu  
Asn170Ser  
Tyr171Ala  
Tyr171Asn  
Tyr171Asp  
Tyr171Cys  
Tyr171Gln  
Tyr171Glu  
Tyr171Gly  
Tyr171His  
Tyr171Ile  
Tyr171Leu



30

Tyr171Met  
 Tyr171Pro  
 Tyr171Ser  
 Tyr171Thr  
 Tyr171Val

TABLE 15

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 Loop 4 - Double Mutation Variants
 

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Ala164Gln + Pro169Glu  
 Asn163Asp + Thr167Ser  
 Ala164Pro + Ala168Asp  
 Ala164Gln + Asn166Ser  
 Gly165Asp + Thr167Pro  
 Ala164Gln + Ala168Ser  
 Gly165Gln + Asn166Gln  
 Gly162Gln + Asn163Glu  
 Gly165Gln + Thr167Glu  
 Gly165Ser + Ala168Gln  
 Ala164Gln + Ala168Glu  
 Ala164His + Tyr171Met  
 Gly165Pro + Pro169Gln  
 Gly165Gln + Ala168Pro  
 Gly162Pro + Ala164Gly  
 Thr167Pro + Asn170Ser  
 Asn163Gln + Tyr171His  
 Asn163Ser + Ala164Gln  
 Ala164Asp + Asn166Gln  
 Gly165Asn + Tyr171Ile  
 Ala168Asp + Asn170Gln  
 Asn166Asp + Ala168Gly  
 Asn163Glu + Thr167Ser  
 Asn166Glu + Ala168Ser  
 Gly162Gln + Ala168Glu  
 Gly162Asn + Asn170Ser  
 Asn163Gln + Ala168His  
 Thr167Pro + Ala168Thr  
 Gly162Asn + Gly165Glu  
 Asn166Glu + Tyr171Pro  
 Ala168Asn + Asn170Gln  
 Gly162Glu + Thr167Ser  
 Thr167Asp + Tyr171Val  
 Gly162Asp + Ala168His  
 Gly165Ser + Ala168Glu  
 Thr167Asp + Tyr171Met  
 Ala168Thr + Pro169Gly  
 Gly165Ser + Pro169Glu  
 Gly162Glu + Ala164Thr  
 Ala168Ser + Tyr171Asp  
 Asn163Ser + Gly165Pro  
 Ala168Asn + Tyr171Ser  
 Ala164Gln + Gly165Gln

Thr167Asp + Asn170Ser  
 Gly165Ser + Asn170Gln  
 Thr167Gly + Tyr171Glu  
 Gly162Asn + Tyr171Gln  
 Gly165Asn + Asn166Glu  
 Ala168Thr + Tyr171Glu  
 Gly165Ser + Asn166Glu  
 Thr167Gly + Ala168Thr  
 Asn166Asp + Ala168Asn  
 Pro169Glu + Tyr171Val  
 Gly162Glu + Asn170Ser  
 Gly162Ser + Pro169Gly  
 Ala164Asn + Asn166Glu  
 Thr167Gly + Asn170Asp  
 Thr167Glu + Asn170Ser  
 Asn163Asp + Ala164Gln  
 Gly165Gln + Pro169Glu

TABLE 16

## Loop 4 - Triple Mutation Variants

Ala164Gly + Gly165Glu + Thr167Ser  
 Gly162Asn + Ala164Thr + Asn166Asp  
 Ala164Asn + Gly165Asp + Pro169Gln  
 Gly165Pro + Thr167Asn + Pro169Glu  
 Ala168Gly + Pro169Gly + Asn170Asp  
 Gly162Asp + Gly165Pro + Ala168Ser  
 Gly165Asp + Thr167Asn + Tyr171Gly  
 Thr167Ser + Pro169Asn + Tyr171Thr  
 Asn166Ser + Ala168Gly + Tyr171Ile  
 Gly165Gln + Thr167Pro + Ala168Glu  
 Gly162Ser + Asn163Asp + Gly165Gln  
 Gly165Glu + Thr167Gly + Ala168Ser  
 Thr167Gly + Ala168Asp + Tyr171Val  
 Asn163Ser + Gly165Gln + Ala168Asp  
 Asn163Asp + Ala164Gly + Gly165Asn  
 Ala164Gln + Pro169Ser + Asn170Gln  
 Ala164His + Thr167Gln + Pro169Gly  
 Thr167Ser + Ala168Asn + Tyr171Asn  
 Gly162Asp + Gly165Ser + Ala168Asn  
 Gly162Pro + Asn163Gln + Tyr171Val  
 Asn166Glu + Ala168Ser + Asn170Gln  
 Thr167Pro + Pro169Ser + Tyr171His  
 Gly162Gln + Asn163Gln + Gly165Asp  
 Ala164Ser + Thr167Ser + Ala168Glu  
 Thr167Gln + Pro169Glu + Asn170Gln  
 Asn163Gln + Pro169Asn + Asn170Gln  
 Gly162Glu + Ala168Gln + Asn170Gln  
 Ala164Gly + Gly165Ser + Asn166Asp  
 Ala164Ser + Ala168Gly + Asn170Gln  
 Asn163Glu + Ala164Asn + Ala168Ser  
 Thr167Ser + Pro169Glu + Tyr171Ser

Ala168Glu + Pro169Gln + Tyr171Ile  
 Gly162Pro + Ala164Pro + Tyr171Pro  
 Gly162Pro + Asn166Ser + Pro169Glu  
 Ala164Gly + Ala168Asn + Tyr171Ser  
 Ala164Thr + Asn170Asp + Tyr171Leu  
 Asn163Gln + Thr167Glu + Pro169Ser  
 Asn166Asp + Ala168Gln + Tyr171Pro  
 Gly162Asn + Ala164Glu + Tyr171Ala  
 Asn166Glu + Thr167Gln + Ala168Pro  
 Ala164Pro + Ala168Gln + Asn170Asp  
 Ala164Thr + Gly165Gln + Ala168Asp  
 Ala164Ser + Ala168Gly + Asn170Glu  
 Ala164His + Gly165Glu + Tyr171Met  
 Ala164Gln + Gly165Asp + Asn166Gln  
 Gly162Gln + Thr167Asn + Ala168Asn  
 Ala168His + Pro169Gln + Tyr171Glu  
 Ala164Gln + Asn166Gln + Tyr171Val  
 Gly162Pro + Ala164Ser + Asn170Gln  
 Thr167Glu + Ala168Gly + Asn170Ser  
 Thr167Ser + Pro169Gly + Asn170Ser  
 Asn163Glu + Asn166Ser + Ala168Ser  
 Asn163Gln + Ala168Glu + Tyr171Ala  
 Thr167Ser + Ala168Ser + Asn170Asp  
 Gly165Asp + Asn166Glu + Tyr171Pro  
 Asn163Asp + Ala164Asp + Thr167Ser  
 Ala164Asp + Gly165Glu + Asn166Gln  
 Gly162Ser + Ala164Glu + Gly165Asp  
 Gly165Asn + Asn170Glu + Tyr171Glu  
 Gly165Pro + Asn170Glu + Tyr171Glu

TABLE 17

## Loop 4 - Quadruple Mutation Variants

Ala164Asn + Asn166Asp + Thr167Asn + Ala168Gln  
 Gly162Asn + Asn163Gln + Ala164Asn + Asn170Glu  
 Ala164Thr + Asn166Ser + Ala168Asn + Pro169Glu  
 Ala164Gln + Pro169Asn + Asn170Glu + Tyr171Thr  
 Asn163Ser + Ala164Thr + Thr167Asp + Tyr171Thr  
 Thr167Ser + Pro169Ser + Asn170Asp + Tyr171Ser  
 Gly162Asn + Thr167Gly + Pro169Gln + Tyr171Ser  
 Gly162Pro + Asn163Ser + Ala168Glu + Tyr171Val  
 Asn163Gln + Ala168Asp + Pro169Gln + Asn170Gln  
 Ala164Asn + Gly165Ser + Pro169Gln + Tyr171Ile  
 Gly162Glu + Thr167Gln + Ala168Ser + Tyr171Ser  
 Gly165Ser + Ala168Gly + Asn170Asp + Tyr171Leu  
 Ala164His + Gly165Asp + Asn166Gln + Tyr171Thr  
 Asn163Gln + Ala164Ser + Asn166Ser + Ala168Glu  
 Asn166Asp + Thr167Asn + Asn170Ser + Tyr171Thr  
 Gly162Asp + Gly165Gln + Asn170Ser + Tyr171Gln  
 Gly162Pro + Ala168Asn + Pro169Glu + Tyr171Gly  
 Asn166Gln + Thr167Gln + Ala168Thr + Asn170Asp  
 Asn163Gln + Thr167Asp + Ala168Ser + Tyr171Pro

Ala164Pro + Thr167Glu + Pro169Ser + Tyr171Val  
 Gly165Asn + Asn166Gln + Pro169Gly + Asn170Asp  
 Asn166Ser + Thr167Pro + Ala168Asn + Asn170Glu  
 Ala164Gly + Ala168Asn + Pro169Asp + Tyr171Ala  
 Ala164Gln + Gly165Gln + Thr167Ser + Pro169Glu  
 Asn163Glu + Gly165Ser + Thr167Gln + Ala168Thr  
 Gly162Pro + Ala164Thr + Thr167Ser + Pro169Gln  
 Gly162Gln + Asn163Glu + Gly165Gln + Thr167Pro  
 Asn163Ser + Pro169Gly + Asn170Glu + Tyr171Ser  
 Asn163Gln + Gly165Ser + Asn170Asp + Tyr171Leu  
 Ala164His + Gly165Pro + Pro169Ser + Asn170Gln  
 Gly162Pro + Asn166Gln + Ala168Asn + Asn170Glu  
 Asn163Gln + Gly165Pro + Thr167Ser + Tyr171Gly  
 Asn163Gln + Gly165Asn + Asn166Ser + Tyr171Cys  
 Asn163Asp + Gly165Pro + Asn166Ser + Tyr171Gly  
 Ala164Pro + Thr167Pro + Pro169Glu + Asn170Ser  
 Asn163Asp + Ala164Glu + Pro169Gln + Tyr171His  
 Asn163Glu + Ala164Glu + Ala168Gly + Tyr171His  
 Thr167Asp + Ala168Glu + Pro169Gln + Asn170Ser  
 Ala164Asp + Gly165Asp + Ala168Asn + Pro169Gln  
 Gly162Ser + Ala164Gly + Asn170Asp + Tyr171Glu  
 Asn163Gln + Thr167Asn + Ala168Asp + Pro169Glu  
 Asn163Ser + Gly165Pro + Ala168Asp + Pro169Glu  
 Gly165Ser + Ala168Asp + Pro169Glu + Asn170Ser  
 Asn166Gln + Ala168Gln + Pro169Asp + Asn170Asp  
 Ala164Gly + Thr167Gly + Pro169Glu + Asn170Asp  
 Gly162Pro + Ala164Gly + Pro169Asp + Asn170Glu  
 Ala164Pro + Thr167Glu + Ala168Asp + Pro169Asp  
 Gly162Asp + Asn163Gln + Ala168His + Asn170Asp  
 Gly162Glu + Ala164Ser + Asn166Ser + Asn170Glu  
 Gly162Glu + Ala164Ser + Asn166Gln + Asn170Glu  
 Thr167Asp + Ala168His + Pro169Glu + Tyr171Pro  
 Asn163Glu + Ala164His + Gly165Asp + Asn166Gln  
 Asn163Asp + Ala164Gln + Gly165Asp + Asn166Ser  
 Asn163Glu + Ala164Asp + Gly165Gln + Ala168Asp  
 Gly162Glu + Ala164Glu + Asn166Ser + Pro169Asp  
 Ala164Glu + Asn166Asp + Asn170Ser + Tyr171Gly  
 Ala164Glu + Gly165Gln + Asn166Glu + Tyr171Cys  
 Ala164Asp + Asn166Glu + Thr167Asn + Pro169Asn  
 Thr167Asp + Pro169Glu + Asn170Glu + Tyr171Ser  
 Asn166Asp + Thr167Ser + Ala168Asp + Tyr171His

TABLE 18

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 Loop 5 - Single Mutation Variants
 

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Ser191Asp  
 Ser191Glu  
 Ser192Asp  
 Ser192Glu  
 Phe193Ala  
 Phe193Asn  
 Phe193Asp

34

Phe193Cys  
 Phe193Gln  
 Phe193Glu  
 Phe193Gly  
 Phe193His  
 Phe193Ile  
 Phe193Leu  
 Phe193Met  
 Phe193Pro  
 Phe193Ser  
 Phe193Thr  
 Phe193Tyr  
 Phe193Val  
 Ser194Asp  
 Ser194Glu  
 Thr195Asn  
 Thr195Asp  
 Thr195Gln  
 Thr195Glu  
 Thr195Gly  
 Thr195Pro  
 Thr195Ser

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TABLE 19

---

 Loop 5 - Double Mutation Variants
 

---

Ser192Asp + Phe193Ser  
 Ser192Asp + Phe193Met  
 Ser191Glu + Phe193Asn  
 Ser191Asp + Phe193Ile  
 Ser191Glu + Phe193Gln  
 Ser191Asp + Thr195Ser  
 Phe193Gly + Thr195Asp  
 Ser192Asp + Phe193Cys  
 Ser191Glu + Thr195Pro  
 Ser191Glu + Phe193Met  
 Phe193Thr + Thr195Glu  
 Ser192Asp + Thr195Gln  
 Phe193Asp + Thr195Asn  
 Ser191Asp + Phe193Gln  
 Phe193Gly + Thr195Pro  
 Ser192Asp + Thr195Ser  
 Ser191Glu + Phe193Ile  
 Phe193Gln + Thr195Asn  
 Ser191Glu + Thr195Gly  
 Ser191Glu + Phe193His  
 Ser191Asp + Phe193Leu  
 Ser192Glu + Phe193Pro  
 Phe193His + Thr195Glu  
 Ser192Asp + Thr195Gly  
 Ser192Glu + Phe193Ala  
 Ser192Glu + Phe193Val

Ser192Asp + Phe193Leu  
 Ser192Glu + Phe193Asn  
 Phe193Tyr + Thr195Ser  
 Phe193His + Thr195Asn  
 Ser192Glu + Thr195Ser  
 Phe193Ile + Thr195Glu  
 Phe193Val + Thr195Asn  
 Phe193Met + Thr195Gln  
 Ser191Asp + Thr195Pro  
 Ser192Glu + Phe193Tyr  
 Phe193Ser + Thr195Asn  
 Ser192Glu + Phe193Gln  
 Ser191Asp + Phe193Ser  
 Ser192Asp + Phe193Ile  
 Ser192Asp + Phe193His  
 Ser192Asp + Phe193Gly  
 Ser191Glu + Phe193Ser  
 Ser192Glu + Thr195Gln  
 Ser191Glu + Thr195Ser  
 Ser191Asp + Thr195Asn  
 Ser191Glu + Phe193Val  
 Phe193Tyr + Thr195Asp  
 Phe193His + Thr195Ser  
 Ser192Glu + Phe193Ile  
 Phe193Met + Thr195Gly  
 Ser191Glu + Phe193Tyr  
 Ser191Asp + Thr195Gln  
 Ser192Asp + Phe193Asn  
 Ser192Asp + Phe193Thr  
 Ser192Glu + Phe193Cys  
 Phe193Asp + Thr195Gln  
 Phe193Val + Thr195Gln  
 Ser192Glu + Phe193Thr  
 Phe193Leu + Thr195Glu

TABLE 20

## Loop 5 - Triple Mutation Variants

Ser192Glu + Phe193Thr + Thr195Gly  
 Ser191Glu + Phe193His + Thr195Ser  
 Ser192Asp + Phe193Leu + Thr195Asn  
 Ser191Asp + Phe193Ala + Thr195Ser  
 Ser192Asp + Phe193Val + Thr195Gln  
 Ser191Asp + Phe193Ser + Thr195Pro  
 Ser191Asp + Phe193Ile + Thr195Gly  
 Ser192Glu + Phe193His + Thr195Gly  
 Ser192Glu + Phe193Leu + Thr195Asn  
 Ser192Asp + Phe193Gly + Thr195Gln  
 Ser192Asp + Phe193Ile + Thr195Pro  
 Ser191Glu + Phe193Asn + Thr195Ser  
 Ser192Asp + Phe193Met + Thr195Asn  
 Ser191Glu + Phe193Gly + Thr195Asn

Ser192Glu + Phe193His + Thr195Asn  
 Ser192Asp + Phe193Met + Thr195Pro  
 Ser191Glu + Phe193Leu + Thr195Asn  
 Ser191Glu + Phe193Met + Thr195Asn  
 Ser192Glu + Phe193Ala + Thr195Gln  
 Ser191Glu + Phe193Tyr + Thr195Gly  
 Ser192Asp + Phe193Ala + Thr195Gly  
 Ser191Glu + Phe193Met + Thr195Gly  
 Ser192Glu + Phe193Thr + Thr195Gln  
 Ser191Asp + Phe193Asn + Thr195Gly  
 Ser192Glu + Phe193Asn + Thr195Ser  
 Ser192Asp + Phe193Ser + Thr195Gly  
 Ser192Glu + Phe193Leu + Thr195Pro  
 Ser191Asp + Phe193Cys + Thr195Gly  
 Ser191Asp + Ser192Asp + Phe193Tyr  
 Ser191Asp + Ser192Asp + Phe193Gly  
 Ser191Asp + Ser192Glu + Phe193Met  
 Ser191Asp + Ser192Glu + Thr195Gln  
 Ser191Glu + Ser192Asp + Phe193Val  
 Ser191Glu + Ser192Glu + Phe193Thr  
 Ser191Glu + Ser192Asp + Phe193Thr  
 Ser191Asp + Ser192Asp + Thr195Gly  
 Ser191Glu + Ser192Glu + Phe193Asn  
 Ser191Asp + Ser192Glu + Phe193Ala  
 Ser191Asp + Ser192Asp + Phe193Asn  
 Ser191Asp + Ser192Asp + Phe193Pro  
 Ser191Asp + Ser192Glu + Phe193Thr  
 Ser191Asp + Ser192Asp + Thr195Asn  
 Ser191Asp + Ser192Asp + Thr195Gln  
 Ser191Glu + Ser192Asp + Phe193Asn  
 Ser191Glu + Ser192Glu + Phe193Gln  
 Ser191Glu + Ser192Asp + Phe193Leu  
 Ser191Asp + Ser192Asp + Thr195Ser  
 Ser191Asp + Ser192Glu + Phe193Ser  
 Ser191Glu + Ser192Asp + Phe193Tyr  
 Ser191Asp + Ser192Asp + Phe193Thr  
 Ser191Asp + Ser192Glu + Phe193Pro  
 Ser191Glu + Ser192Asp + Phe193Ile  
 Ser191Asp + Ser192Glu + Phe193Tyr  
 Ser191Glu + Ser192Glu + Phe193Tyr  
 Ser191Asp + Ser192Asp + Phe193Ser  
 Ser191Glu + Ser192Glu + Thr195Pro  
 Ser191Asp + Ser192Glu + Thr195Ser  
 Ser191Glu + Ser192Asp + Phe193Met  
 Ser191Asp + Ser192Glu + Thr195Pro  
 Ser191Glu + Ser192Asp + Phe193His

TABLE 21

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 Loop 5 - Quadruple Mutation Variants
 

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Ser191Asp + Ser192Glu + Phe193Gly + Thr195Gln  
 Ser191Glu + Ser192Asp + Phe193His + Thr195Gly

Ser191Glu + Ser192Glu + Phe193Cys + Thr195Pro  
Ser191Asp + Ser192Glu + Phe193Cys + Thr195Gln  
Ser191Asp + Ser192Glu + Phe193Thr + Thr195Pro  
Ser191Glu + Ser192Glu + Phe193Val + Thr195Asn  
Ser191Asp + Ser192Glu + Phe193Val + Thr195Asn  
Ser191Glu + Ser192Glu + Phe193Leu + Thr195Ser  
Ser191Glu + Ser192Asp + Phe193Cys + Thr195Gln  
Ser191Glu + Ser192Asp + Phe193Pro + Thr195Pro  
Ser191Glu + Ser192Asp + Phe193Ser + Thr195Gly  
Ser191Glu + Ser192Asp + Phe193Pro + Thr195Asn  
Ser191Glu + Ser192Glu + Phe193Ser + Thr195Pro  
Ser191Glu + Ser192Asp + Phe193Ala + Thr195Gly  
Ser191Asp + Ser192Glu + Phe193His + Thr195Asn  
Ser191Asp + Ser192Glu + Phe193Asn + Thr195Asn  
Ser191Glu + Ser192Glu + Phe193Gln + Thr195Ser  
Ser191Glu + Ser192Glu + Phe193Thr + Thr195Gln  
Ser191Glu + Ser192Glu + Phe193Asn + Thr195Gln  
Ser191Glu + Ser192Glu + Phe193Tyr + Thr195Ser  
Ser191Asp + Ser192Glu + Phe193Ile + Thr195Pro  
Ser191Glu + Ser192Asp + Phe193Val + Thr195Gln  
Ser191Glu + Ser192Asp + Phe193Val + Thr195Asn  
Ser191Asp + Ser192Glu + Phe193Tyr + Thr195Ser  
Ser191Asp + Ser192Glu + Phe193Ala + Thr195Ser  
Ser191Glu + Ser192Glu + Phe193His + Thr195Asn  
Ser191Asp + Ser192Asp + Phe193Pro + Thr195Asn  
Ser191Asp + Ser192Asp + Phe193Gln + Thr195Ser  
Ser191Asp + Ser192Asp + Phe193Leu + Thr195Asn  
Ser191Asp + Ser192Glu + Phe193Val + Thr195Gln  
Ser191Glu + Ser192Glu + Phe193Gly + Thr195Gly  
Ser191Glu + Ser192Glu + Phe193Thr + Thr195Pro  
Ser191Glu + Ser192Glu + Phe193Thr + Thr195Gly  
Ser191Glu + Ser192Asp + Phe193Ile + Thr195Ser  
Ser191Glu + Ser192Glu + Phe193Ile + Thr195Gly  
Ser191Asp + Ser192Asp + Phe193Pro + Thr195Pro  
Ser191Asp + Ser192Asp + Phe193Ile + Thr195Gly  
Ser191Glu + Ser192Asp + Phe193Thr + Thr195Gly  
Ser191Asp + Ser192Glu + Phe193Ser + Thr195Gly  
Ser191Glu + Ser192Asp + Phe193Tyr + Thr195Ser  
Ser191Asp + Ser192Asp + Phe193Thr + Thr195Gln  
Ser191Asp + Ser192Glu + Phe193Ser + Thr195Gln  
Ser191Glu + Ser192Glu + Phe193His + Thr195Ser  
Ser191Glu + Ser192Asp + Phe193Leu + Thr195Asn  
Ser191Asp + Ser192Glu + Phe193Leu + Thr195Pro  
Ser191Asp + Ser192Asp + Phe193Ile + Thr195Pro  
Ser191Asp + Ser192Asp + Phe193Tyr + Thr195Ser  
Ser191Asp + Ser192Glu + Phe193Ile + Thr195Gln  
Ser191Asp + Ser192Glu + Phe193Pro + Thr195Ser  
Ser191Asp + Ser192Glu + Phe193Leu + Thr195Gln  
Ser191Glu + Ser192Asp + Phe193Asn + Thr195Ser  
Ser191Glu + Ser192Glu + Phe193Ser + Thr195Asn  
Ser191Asp + Ser192Asp + Phe193Glu + Thr195Gln  
Ser191Asp + Ser192Glu + Phe193Glu + Thr195Pro  
Ser191Glu + Ser192Asp + Phe193Glu + Thr195Gln



Ser191Glu + Ser192Glu + Phe193Glu + Thr195Gly  
 Ser191Glu + Ser192Glu + Phe193Asp + Thr195Gly  
 Ser191Asp + Ser192Asp + Phe193Glu + Thr195Ser  
 Ser191Glu + Ser192Asp + Phe193Asp + Thr195Pro  
 Ser191Glu - Ser192Glu + Phe193Asp + Thr195Gln

TABLE 22

## Loop 6 - Single Mutation Variants

Ala204Asn  
 Ala204Asp  
 Ala204Gln  
 Ala204Glu  
 Ala204Gly  
 Ala204His  
 Ala204Pro  
 Ala204Ser  
 Ala204Thr  
 Pro205Asn  
 Pro205Asp  
 Pro205Gln  
 Pro205Glu  
 Pro205Gly  
 Pro205Ser  
 Gly206Asn  
 Gly206Asp  
 Gly206Gln  
 Gly206Glu  
 Gly206Pro  
 Gly206Ser  
 Ser207Asp  
 Ser207Glu  
 Trp208Ala  
 Trp208Asn  
 Trp208Asp  
 Trp208Cys  
 Trp208Gln  
 Trp208Glu  
 Trp208Gly  
 Trp208His  
 Trp208Ile  
 Trp208Leu  
 Trp208Met  
 Trp208Phe  
 Trp208Pro  
 Trp208Ser  
 Trp208Thr  
 Trp208Tyr  
 Trp208Val  
 Ile209Ala  
 Ile209Asn  
 Ile209Asp

Ile209Cys  
Ile209Gln  
Ile209Glu  
Ile209Gly  
Ile209His  
Ile209Leu  
Ile209Met  
Ile209Pro  
Ile209Ser  
Ile209Thr  
Ile209Val  
Tyr210Ala  
Tyr210Asn  
Tyr210Asp  
Tyr210Cys  
Tyr210Gln  
Tyr210Glu  
Tyr210Gly  
Tyr210His  
Tyr210Ile  
Tyr210Leu  
Tyr210Met  
Tyr210Pro  
Tyr210Ser  
Tyr210Thr  
Tyr210Val  
Ser211Asp  
Ser211Glu  
Thr212Asn  
Thr212Asp  
Thr212Gln  
Thr212Glu  
Thr212Gly  
Thr212Pro  
Thr212Ser  
Tyr213Ala  
Tyr213Asn  
Tyr213Asp  
Tyr213Cys  
Tyr213Gln  
Tyr213Glu  
Tyr213Gly  
Tyr213His  
Tyr213Ile  
Tyr213Leu  
Tyr213Met  
Tyr213Pro  
Tyr213Ser  
Tyr213Thr  
Tyr213Val  
Pro214Asn  
Pro214Asp  
Pro214Gln

Pro214Glu  
Pro214Gly  
Pro214Ser  
Thr215Asn  
Thr215Asp  
Thr215Gln  
Thr215Glu  
Thr215Gly  
Thr215Pro  
Thr215Ser  
Ser216Asp  
Ser216Glu  
Thr217Asn  
Thr217Asp  
Thr217Gln  
Thr217Glu  
Thr217Gly  
Thr217Pro  
Thr217Ser  
Tyr218Ala  
Tyr218Asn  
Tyr218Asp  
Tyr218Cys  
Tyr218Gln  
Tyr218Glu  
Tyr218Gly  
Tyr218His  
Tyr218Ile  
Tyr218Leu  
Tyr218Met  
Tyr218Pro  
Tyr218Ser  
Tyr218Thr  
Tyr218Val  
Ala219Asn  
Ala219Asp  
Ala219Gln  
Ala219Glu  
Ala219Gly  
Ala219His  
Ala219Pro  
Ala219Ser  
Ala219Thr  
Ser220Asp  
Ser220Glu  
Leu221Ala  
Leu221Asn  
Leu221Asp  
Leu221Cys  
Leu221Gln  
Leu221Glu  
Leu221Gly  
Leu221His

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Leu221Ile  
 Leu221Met  
 Leu221Pro  
 Leu221Ser  
 Leu221Thr  
 Leu221Val  
 Ser222Asp  
 Ser222Glu  
 Gly223Asn  
 Gly223Asp  
 Gly223Gln  
 Gly223Glu  
 Gly223Pro  
 Gly223Ser  
 Thr224Asn  
 Thr224Asp  
 Thr224Gln  
 Thr224Glu  
 Thr224Gly  
 Thr224Pro  
 Thr224Ser

TABLE 23

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 Loop 6 - Double Mutation Variants
 

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Ser207Glu + Thr215Gln  
 Pro205Gly + Ala219Gly  
 Thr217Asn + Thr224Gly  
 Gly206Gln + Pro214Asp  
 Ala204Asn + Thr212Ser  
 Pro205Gly + Ser220Asp  
 Tyr213Gln + Tyr218Glu  
 Pro205Gln + Leu221Glu  
 Thr212Gln + Ser220Asp  
 Tyr210Gly + Thr224Gly  
 Tyr213Gly + Ser216Glu  
 Ser207Glu + Gly223Asn  
 Tyr210Ser + Tyr218Leu  
 Ser216Glu + Thr217Ser  
 Ser216Glu + Thr224Asn  
 Pro205Asn + Ala219Asn  
 Thr217Gly + Leu221Thr  
 Thr215Glu + Ala219Gln  
 Thr217Asn + Tyr218Glu  
 Ala204Asn + Gly206Pro  
 Thr212Ser + Thr224Gln  
 Thr217Gly + Ser222Glu  
 Trp208Glu + Ile209Asn  
 Pro205Ser + Ala219Gly  
 Tyr213Ser + Ala219Ser  
 Ala204Ser + Gly206Ser  
 Ala204Gly + Thr215Glu

Thr212Gln + Ala219Glu  
Ala204Gly + Trp208Gln  
Ala204Thr + Ser216Asp  
Thr212Gln + Tyr218Gln  
Ser207Glu + Leu221Met  
Thr215Pro + Gly223Gln  
Ala204Gly + Ser216Asp  
Tyr210Asp + Ala219His  
Ile209Gln + Tyr218Ala  
Ile209Ala + Ala219Pro  
Ala204Asn + Ser222Glu  
Tyr210Asp + Tyr213Ala  
Tyr218Leu + Thr224Glu  
Tyr218Asn + Ser220Glu  
Trp208Met + Ala219Pro  
Pro205Gly + Ser222Asp  
Leu221Val + Thr224Gln  
Trp208Ile + Pro214Ser  
Trp208Asp + Thr212Pro  
Ala204Thr + Gly223Gln  
Thr217Gln + Tyr218Ile  
Tyr213His + Ser216Asp  
Thr212Ser + Thr215Asp  
Thr217Asn + Leu221Asn  
Pro214Asp + Thr217Pro  
Ser207Glu + Ala219His  
Thr217Asp + Tyr218His  
Trp208Ala + Thr217Ser  
Pro205Gln + Ser220Asp  
Ile209Gly + Ser216Glu  
Tyr218Ser + Ser220Glu  
Thr215Asn + Ser216Glu  
Thr215Gly + Tyr218Gln  
Thr217Ser + Leu221Ala  
Ala219Pro + Leu221His  
Pro205Gln + Leu221Met  
Tyr213Met + Leu221Ile  
Trp208Asn + Thr212Ser  
Ser207Glu + Thr224Gly  
Pro205Ser + Leu221Cys  
Ala204Gly + Tyr210Val  
Trp208Phe + Ser222Asp  
Ile209Thr + Ser222Glu  
Leu221Ile + Ser222Glu  
Tyr213Pro + Thr217Glu  
Ile209Thr + Ser216Glu  
Tyr210Cys + Ser222Asp  
Leu221Glu + Thr224Ser  
Tyr213Ala + Thr217Pro  
Trp208Cys + Thr215Glu  
Ala204Ser + Thr217Ser  
Ile209His + Gly223Ser  
Ala204Asn + Trp208Tyr

Pro205Gln + Ser207Glu  
Ala204Asn + Trp208Met  
Tyr210Pro + Leu221Asp  
Tyr210Gln + Thr215Ser  
Ala204Pro + Ser216Glu  
Ala204Gly + Ser222Asp  
Ser216Asp + Leu221Met  
Tyr210Ile + Ala219Asn  
Pro214Glu + Thr224Ser  
Pro205Gln + Thr215Ser  
Ala204Gly + Pro205Asn  
Tyr210Asp + Tyr218Val  
Trp208Ser + Thr215Gly  
Ser222Glu + Thr224Ser  
Trp208Gln + Ser216Asp  
Ser207Asp + Gly223Ser  
Pro205Gly + Tyr210Cys  
Thr212Gly + Thr224Asp  
Ser220Glu + Gly223Pro  
Gly206Pro + Tyr210Met  
Tyr210Ser + Ser222Asp  
Ser207Glu + Pro214Gln  
Pro205Gly + Gly223Asn  
Tyr213Ser + Pro214Asp  
Pro214Gln + Ser220Asp  
Ala204Thr + Ser222Glu  
Ala219Asn + Leu221Asp  
Gly206Ser + Thr215Pro  
Thr212Asn + Tyr218Val  
Thr217Pro + Gly223Asp  
Gly206Pro + Tyr218Asn  
Gly206Gln + Thr217Asp  
Tyr210Ala + Ser220Asp  
Pro214Asp + Tyr218Val  
Leu221His + Gly223Asp  
Pro214Gln + Gly223Asp  
Ile209Pro + Ser216Asp  
Ile209Thr + Gly223Asp  
Pro214Asn + Ala219Asp  
Tyr218Cys + Ser220Glu  
Ser207Glu + Tyr218Asn  
Tyr218Gly + Leu221Gln  
Ile209Thr + Tyr218Val  
Pro214Glu + Gly223Ser  
Tyr213Met + Ala219Thr  
Ser222Glu + Thr224Asn  
Trp208Asp + Ile209Met  
Thr212Gln + Pro214Gln  
Ser207Asp + Tyr213Leu  
Thr212Gln + Gly223Pro  
Tyr210Leu + Ser216Glu  
Ser216Asp + Ala219Thr  
Tyr213Gln + Thr217Asp

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Gly206Pro + Ser222Glu  
 Ala204Thr + Ser220Asp  
 Ala219Thr + Thr224Pro  
 Trp208Ile + Ala219Glu  
 Ile209Gly + Thr215Ser  
 Gly206Gln + Tyr210Glu  
 Pro214Gln + Ser220Glu  
 Ser207Glu + Thr217Asn  
 Ala204Pro + Gly223Asp  
 Ile209Cys + Ser222Asp  
 Pro214Gly + Gly223Pro  
 Ile209Met + Thr224Pro  
 Pro214Glu + Thr217Ser  
 Pro205Ser + Ser207Asp  
 Tyr210Thr + Thr212Gln  
 Gly206Gln + Tyr218Pro  
 Tyr218Val + Ser222Glu  
 Leu221Asn + Gly223Glu  
 Pro205Gln + Tyr218Val  
 Pro205Gln + Thr217Asn  
 Trp208His + Ile209Ala  
 Pro205Ser + Gly223Gln  
 Pro214Ser + Thr217Gly  
 Tyr218His + Ser222Asp  
 Thr212Gln + Gly223Ser  
 Thr215Glu + Ala219Thr  
 Tyr213Gly + Tyr218Asp  
 Thr212Gln + Tyr213Gln  
 Tyr210Ile + Thr215Asp  
 Ala204Asn + Gly223Pro  
 Ser207Glu + Ile209Asn  
 Thr215Gln + Ala219Asp  
 Ser220Asp + Gly223Pro  
 Pro205Ser + Tyr213Ala  
 Trp208Gly + Ser222Glu  
 Thr215Glu + Tyr218Ala  
 Ala204Gln + Ala219Gln  
 Pro205Asn + Tyr213Thr  
 Tyr218Thr + Thr224Gln  
 Ser222Asp + Thr224Ser  
 Ala204Pro + Tyr213Cys  
 Gly206Gln + Leu221Gly  
 Ser216Glu + Thr224Ser  
 Ser207Asp + Ala219Asn  
 Trp208Leu + Ile209His  
 Pro205Ser + Tyr213Val  
 Thr212Gly + Thr215Asp

TABLE 24

## Loop 6 - Triple Mutation Variants

Gly206Ser + Thr212Ser + Ala219Glu

Ala204Asn + Thr212Ser + Ser216Glu  
Pro205Gln + Leu221Glu + Gly223Asn  
Trp208Thr + Ile209Cys + Thr224Asp  
Ile209Met + Thr212Gln + Ser220Asp  
Tyr210Gly + Tyr213Gly + Thr224Gly  
Ser207Glu + Tyr210Ser + Gly223Asn  
Tyr213Gly + Ser216Asp + Thr217Ser  
Pro205Asn + Ser216Glu + Thr224Asn  
Thr215Glu + Ala219Gln + Leu221Thr  
Ala204Asn + Thr217Asn + Tyr218Glu  
Thr212Ser + Thr217Gly + Thr224Gln  
Ala204Ser + Tyr218His + Ala219Gln  
Pro205Ser + Tyr213Ser + Leu221Asn  
Ala204Ser + Gly206Ser + Ala219Ser  
Ala204Gly + Trp208Gln + Ser216Asp  
Ser207Glu + Tyr218Gln + Leu221Met  
Ala204Gly + Thr215Pro + Gly223Gln  
Ile209Gln + Tyr218Ala + Ala219Pro  
Ala204Asn + Trp208Met + Ser222Glu  
Trp208Met + Ala219Pro + Ser222Asp  
Pro205Gly + Leu221Val + Thr224Gln  
Ala204Thr + Thr212Pro + Gly223Gln  
Gly206Gln + Ile209Gln + Thr212Gln  
Gly206Pro + Ser220Glu + Leu221Ser  
Gly206Gln + Tyr210Gly + Ser222Asp  
Ala204Gly + Tyr213Thr + Pro214Gln  
Ala204His + Thr212Gln + Thr224Gln  
Gly206Gln + Pro214Asp + Tyr218Ser  
Thr212Asn + Ser216Asp + Thr217Ser  
Ser207Asp + Tyr213Ile + Thr224Pro  
Pro205Gln + Tyr213Met + Leu221His  
Trp208Asn + Thr212Ser + Gly223Asp  
Ser207Glu + Leu221Gly + Thr224Gly  
Pro205Ser + Tyr210Val + Leu221Cys  
Pro205Gly + Ile209His + Tyr213Thr  
Ile209Pro + Tyr210Asn + Ser220Asp  
Pro205Gly + Thr212Pro + Thr224Gly  
Trp208Cys + Thr217Asp + Ala219Asn  
Pro205Gly + Leu221Cys + Gly223Pro  
Ala204His + Ser216Glu + Leu221Ser  
Thr217Asp + Gly223Ser + Thr224Gly  
Pro205Gln + Trp208Gln + Ile209Ala  
Thr212Gly + Thr217Gly + Leu221His  
Gly206Asn + Thr212Gly + Tyr213Gly  
Gly206Asn + Ser207Glu + Thr212Ser  
Thr212Gln + Leu221Glu + Gly223Ser  
Tyr210His + Thr215Asp + Thr224Pro  
Pro214Gln + Thr215Pro + Ser216Glu  
Pro205Gly + Tyr213Val + Gly223Asp  
Trp208Phe + Thr215Asn + Thr224Gly  
Tyr213Met + Ala219Pro + Ser220Glu  
Trp208Phe + Ile209Ser + Tyr213Gln  
Gly206Ser + Ser216Glu + Tyr218Pro



Ala204Gln + Tyr210His + Thr224Glu  
Pro205Ser + Thr212Pro + Ala219Ser  
Pro205Gln + Tyr210Met + Thr217Pro  
Ala204Gly + Ile209His + Ser216Glu  
Thr212Asn + Thr217Ser + Thr224Asn  
Gly206Pro + Tyr210Gly + Gly223Gln  
Pro205Gln + Thr217Asp + Ala219Thr  
Pro205Ser + Ile209Met + Ser216Asp  
Thr215Asn + Ser216Glu + Thr224Ser  
Pro205Gln + Leu221Thr + Ser222Glu  
Tyr210Cys + Ala219Asn + Leu221Thr  
Ala204Ser + Gly206Ser + Ala219Gln  
Ile209Pro + Ser216Asp + Leu221Cys  
Trp208Thr + Tyr210Val + Thr212Ser  
Thr212Gly + Pro214Asp + Gly223Gln  
Pro214Gln + Thr215Ser + Thr224Glu  
Thr212Ser + Pro214Gln + Leu221His  
Trp208Gly + Ser220Glu + Gly223Ser  
Pro205Ser + Ser220Asp + Leu221Thr  
Gly206Asn + Thr215Pro + Leu221Ala  
Tyr213Leu + Leu221His + Gly223Asp  
Ser207Asp + Thr212Gly + Tyr218Cys  
Gly206Gln + Ile209Val + Ala219Thr  
Trp208Ile + Tyr218Ile + Gly223Asn  
Ala204His + Ile209Gly + Ser216Asp  
Trp208Leu + Thr212Gly + Ala219Ser  
Thr212Gln + Pro214Glu + Ala219Thr  
Pro205Gln + Ser207Asp + Thr212Pro  
Pro205Ser + Tyr210Pro + Tyr218Ile  
Leu221Ala + Gly223Asn + Thr224Glu  
Ala204Thr + Gly206Gln + Ser207Asp  
Ser216Glu + Ala219Gln + Gly223Gln  
Pro205Gly + Trp208Leu + Leu221Gln  
Thr217Gln + Gly223Gln + Thr224Glu  
Thr212Gly + Thr217Ser + Tyr218Ala  
Gly206Asn + Gly223Asp + Thr224Asn  
Ala204Gly + Tyr210Cys + Tyr213Thr  
Gly206Ser + Thr212Asn + Thr215Gly  
Ala204Asn + Thr212Asn + Ser216Glu  
Gly206Gln + Tyr218Cys + Gly223Glu  
Ala204Ser + Pro205Ser + Gly206Asn  
Ile209Gly + Leu221Val + Thr224Pro  
Gly206Asn + Trp208His + Thr217Gly  
Gly206Ser + Trp208Leu + Ser222Asp  
Pro205Gly + Thr212Gln + Ser222Asp  
Ala204Thr + Tyr213Gln + Thr224Pro  
Thr212Ser + Thr217Ser + Gly223Asp  
Pro205Ser + Tyr213Ile + Ser222Glu  
Ile209Pro + Tyr210Asp + Ala219Pro  
Gly206Gln + Thr215Pro + Ser220Glu  
Trp208Cys + Tyr210Leu + Thr215Gly  
Trp208Tyr + Thr212Gln + Ser222Glu  
Gly206Asn + Ser222Asp + Gly223Asn

Pro205Gly + Gly206Asn + Leu221Cys  
Pro205Gln + Tyr213Ile + Ser216Glu  
Ala204His + Tyr210His + Ser220Asp  
Ala204Thr + Thr212Asn + Ala219Asp  
Ser207Glu + Tyr210Ala + Thr212Gly  
Tyr210His + Thr217Gly + Leu221Met  
Ala204Thr + Ile209Met + Ser222Glu  
Trp208Pro + Ala219Pro + Ser222Asp  
Ala204His + Ile209Ser + Ser220Glu  
Pro214Ser + Leu221Glu + Gly223Gln  
Gly206Pro + Leu221Met + Gly223Asp  
Ala204His + Tyr213Thr + Ser222Asp  
Tyr213Ala + Leu221Met + Ser222Asp  
Trp208Leu + Thr212Ser + Pro214Asp  
Tyr213Ile + Ser216Glu + Thr217Pro  
Pro205Gln + Pro214Gln + Thr224Gly  
Tyr213Cys + Thr217Gly + Ser220Glu  
Gly206Asn + Thr212Gly + Tyr218Pro  
Gly206Pro + Ser216Glu + Gly223Pro  
Thr212Gln + Thr215Gly + Ser222Glu  
Ala204Pro + Thr217Asp + Thr224Ser  
Pro205Ser + Thr212Pro + Ala219Asp  
Ile209Cys + Pro214Ser + Thr215Asp  
Ile209Cys + Ser220Asp + Gly223Gln  
Thr217Gly + Ser220Asp + Leu221Gln  
Ala204His + Pro205Asn + Trp208Glu  
Ala204Gln + Thr212Ser + Thr215Asp  
Pro214Asn + Ser220Glu + Leu221Gly  
Ile209Gly + Thr215Asn + Ala219Pro  
Ser207Glu + Thr217Gln + Thr224Ser  
Ala204Gly + Thr215Asn + Gly223Pro  
Ile209Ser + Tyr213Ile + Leu221Met  
Tyr210Thr + Pro214Ser + Ser222Asp  
Tyr210Leu + Thr212Ser + Leu221Asn  
Gly206Asn + Pro214Asp + Tyr218Met  
Gly206Asn + Pro214Gln + Ala219Asn  
Ser207Glu + Pro214Ser + Ala219His  
Ala204Asn + Trp208Ala + Ile209Gln  
Thr217Glu + Tyr218Leu + Gly223Asn  
Trp208Pro + Pro214Gly + Leu221Ala  
Gly206Pro + Thr215Ser + Leu221Cys  
Ala204Ser + Ile209Val + Thr224Asp  
Trp208Ile + Tyr210Pro + Thr224Asp  
Thr212Gln + Thr215Asn + Thr217Asp  
Tyr210Ser + Tyr213Cys + Thr217Asn  
Thr212Gln + Ser222Asp + Gly223Gln  
Tyr210Asn + Thr217Glu + Tyr218Met  
Pro214Asn + Ala219Gln + Ser220Glu  
Pro205Ser + Trp208Met + Leu221Asp  
Ala204Asn + Tyr210Cys + Tyr213Asn  
Tyr218Leu + Leu221His + Ser222Glu  
Ala204His + Tyr218Ser + Ser222Asp  
Gly206Pro + Tyr213Asn + Pro214Asn

Gly206Pro + Thr215Gln + Tyr218Ser  
 Ala204Pro + Ile209His + Leu221Thr  
 Gly206Gln + Thr212Gly + Gly223Glu  
 Thr215Gly + Tyr218Ala + Thr224Pro  
 Pro205Gln + Tyr213Gly + Gly223Glu  
 Ala204Gly + Tyr213Asn + Leu221Thr  
 Trp208Gly + Ile209His + Thr215Ser  
 Ala204His + Ser207Glu + Tyr213Cys  
 Trp208His + Tyr210His + Ser222Asp  
 Pro205Gly + Tyr210Asp + Leu221Gln  
 Pro205Asn + Tyr210Ala + Pro214Ser  
 Thr212Pro + Thr215Gln + Thr224Asp  
 Ala204Thr + Tyr213Val + Pro214Glu  
 Ala204Gly + Tyr210Pro + Pro214Glu  
 Pro205Gly + Trp208His + Tyr218Gly  
 Gly206Ser + Ala219Gln + Gly223Ser  
 Tyr213Thr + Tyr218Cys + Ser220Glu  
 Thr215Pro + Ser216Glu + Leu221Asn  
 Pro205Gly + Thr212Asn + Thr224Asp  
 Pro205Gly + Ser207Asp + Thr212Ser

TABLE 25

## Loop 6 - Quadruple Mutation Variants

Gly206Asn + Thr215Gln + Tyr218Ile + Leu221Ile  
 Tyr210Gly + Thr212Gln + Tyr213Gly + Thr224Gly  
 Pro205Gly + Gly206Pro + Trp208Asn + Thr224Ser  
 Ile209Thr + Pro214Asp + Thr217Asn + Tyr218Cys  
 Ala204Ser + Tyr218His + Ala219Gln + Leu221Asn  
 Ala204Ser + Pro205Ser + Tyr213Ser + Ala219Ser  
 Ala204Gly + Thr212Gln + Ser216Asp + Gly223Ser  
 Ser207Glu + Thr215Pro + Tyr218Gln + Leu221Met  
 Ala204Gly + Ser216Asp + Ala219His + Gly223Gln  
 Ile209Gln + Tyr210Asp + Tyr218Ala + Ala219Pro  
 Trp208Met + Tyr218Leu + Ala219Pro + Ser222Glu  
 Pro205Gly + Leu221Val + Ser222Asp + Thr224Gln  
 Ala204Thr + Thr212Pro + Ser222Glu + Gly223Gln  
 Gly206Gln + Ile209Gln + Thr212Gln + Leu221His  
 Ala204Ser + Gly206Pro + Ser220Asp + Leu221Ser  
 Ala204Gly + Thr212Gln + Tyr213Thr + Thr224Gln  
 Tyr210Ala + Pro214Ser + Tyr218Val + Ser222Asp  
 Ala204Gln + Pro205Ser + Gly206Ser + Ser222Glu  
 Trp208Cys + Pro214Gln + Ala219Thr + Thr224Asp  
 Ala204Gly + Gly206Ser + Tyr210Asp + Leu221Gly  
 Ala204Gln + Gly206Asn + Tyr210Gln + Ala219Asp  
 Pro205Ser + Gly206Pro + Ile209Val + Tyr213Pro  
 Gly206Pro + Pro214Glu + Thr215Pro + Leu221Met  
 Ser207Asp + Trp208Met + Tyr218Val + Leu221Pro  
 Tyr210Gly + Thr212Pro + Pro214Gly + Ser222Asp  
 Gly206Pro + Thr212Gln + Thr217Ser + Ser222Asp  
 Ala204Pro + Trp208Met + Ile209Val + Ser216Glu  
 Trp208Ser + Tyr218His + Ala219His + Gly223Pro

Ala204Gln + Trp208Ala + Tyr210Asn + Ser222Glu  
Gly206Gln + Ser220Asp + Leu221Ala + Thr224Ser  
Ile209Gln + Thr212Ser + Ala219Gln + Ser220Glu  
Pro205Asn + Thr212Gly + Tyr218Ala + Ser222Glu  
Pro205Gln + Trp208Cys + Tyr213Cys + Pro214Asp  
Pro205Gly + Thr215Gln + Thr217Pro + Gly223Ser  
Ala204His + Gly206Pro + Ser216Glu + Leu221Ser  
Ser207Asp + Leu221Gly + Gly223Gln + Thr224Asn  
Tyr210His + Thr212Ser + Leu221Glu + Gly223Ser  
Pro205Gly + Thr215Asn + Gly223Asp + Thr224Gly  
Trp208Phe + Tyr213Met + Thr215Pro + Ser220Glu  
Gly206Ser + Trp208Phe + Ile209Ser + Tyr213Gln  
Ala204Gln + Pro205Ser + Thr212Pro + Ala219Ser  
Ala204Gly + Tyr210Met + Ser216Glu + Thr217Pro  
Gly206Pro + Thr212Asn + Thr217Gln + Thr224Asn  
Pro205Ser + Ile209Met + Thr215Asn + Thr224Ser  
Tyr210Thr + Thr212Pro + Tyr218Asn + Ser220Glu  
Trp208Thr + Tyr210Val + Thr212Ser + Leu221Cys  
Thr212Gly + Pro214Asp + Thr215Ser + Gly223Gln  
Ser207Glu + Thr212Ser + Pro214Gln + Leu221Pro  
Pro205Asn + Gly206Gln + Ser207Asp + Leu221Thr  
Pro205Ser + Thr215Pro + Ser220Asp + Leu221Cys  
Gly206Asn + Tyr213Leu + Leu221Ala + Gly223Asp  
Ser207Asp + Thr212Gly + Pro214Asn + Thr224Asn  
Ala204Pro + Ser207Glu + Ile209Val + Ala219Thr  
Gly206Gln + Trp208Ile + Tyr218Ile + Gly223Asn  
Ala204His + Trp208Leu + Ile209Gly + Ser216Asp  
Ala204Gln + Thr212Gly + Pro214Glu + Ala219Ser  
Ala204Asn + Pro205Gln + Ser207Asp + Thr212Pro  
Pro205Gly + Trp208Leu + Ser216Glu + Ala219Gln  
Trp208Tyr + Thr217Gln + Leu221Gln + Thr224Glu  
Thr212Gly + Thr217Ser + Tyr218Ala + Gly223Asp  
Ala204Gly + Gly206Asn + Thr215Asn + Gly223Asp  
Gly206Ser + Tyr210Cys + Tyr213Thr + Thr215Gly  
Ala204Gln + Pro205Ser + Gly206Asn + Ala219Asp  
Ala204Ser + Ile209Gly + Leu221Val + Thr224Pro  
Gly206Asn + Ser207Glu + Trp208His + Thr217Gly  
Gly206Ser + Trp208Pro + Ala219Thr + Ser222Asp  
Ala204Thr + Tyr213Gln + Thr217Ser + Thr224Pro  
Pro205Ser + Thr212Ser + Tyr213Ile + Gly223Asp  
Gly206Gln + Ile209Asn + Thr215Pro + Ser220Glu  
Trp208Cys + Thr212Gln + Thr215Gly + Ser222Glu  
Gly206Asn + Trp208Tyr + Ser222Glu + Gly223Asn  
Pro205Gly + Gly206Asn + Leu221Cys + Ser222Asp  
Pro205Gln + Tyr210His + Tyr213Ile + Ser220Asp  
Ala204His + Trp208Asn + Thr212Asn + Ala219Asp  
Ala204His + Gly206Pro + Leu221Met + Gly223Asp  
Ala204Ser + Tyr213Thr + Leu221Met + Ser222Asp  
Tyr213Ile + Pro214Gln + Ser216Glu + Thr217Pro  
Pro205Gln + Tyr213Cys + Ser220Glu + Thr224Gly  
Gly206Asn + Tyr213Asn + Tyr218Pro + Ser220Glu  
Pro205Ser + Gly206Pro + Tyr218Asp + Gly223Pro  
Ala204Pro + Pro205Ser + Thr217Asp + Thr224Ser

Pro205Asn - Trp208Ile + Thr212Gln + Thr217Gly  
Ile209Cys + Pro214Ser + Ser220Asp + Gly223Gln  
Ala204His - Pro205Asn + Trp208Glu + Tyr210His  
Ile209Gly + Thr215Asn + Thr217Asn + Ala219Pro  
Pro205Gly - Pro214Asn + Tyr218Ile + Ser222Asp  
Ala204Ser + Pro205Ser + Thr212Asn + Tyr213Ile  
Pro205Gln + Ser207Glu + Tyr210Pro + Thr215Ser  
Pro205Gln + Ser207Glu + Thr212Gln + Tyr213Ser  
Ala204Ser - Ile209Asn + Tyr213Cys + Ser216Asp  
Trp208Ala + Ile209Gln + Thr217Glu + Gly223Asn  
Trp208Pro + Pro214Gly + Tyr218Leu + Leu221Ala  
Gly206Pro + Thr215Ser + Ser216Glu + Leu221Cys  
Ala204Ser + Trp208Ile + Ile209Val + Thr224Asp  
Tyr210Pro + Thr212Gln + Thr215Asn + Thr217Asp  
Tyr210Ser + Tyr213Cys + Pro214Asn + Thr217Asn  
Thr212Gln + Tyr218Met + Ser222Asp + Gly223Gln  
Pro205Ser + Trp208Met + Ala219Gln + Leu221Asp  
Ala204Asn + Tyr210Cys + Tyr213Asn + Tyr218Leu  
Ala204His + Pro205Gly + Tyr218Ser + Ser222Asp  
Gly206Pro + Tyr213Asn + Pro214Asn + Tyr218Ser  
Ala204Pro + Ile209His + Thr215Gln + Leu221Thr  
Ala204Asn + Gly206Gln + Thr212Gly + Gly223Glu  
Thr215Gly + Tyr218Ala + Gly223Glu + Thr224Pro  
Pro205Gln + Tyr213Gly + Ser220Asp + Leu221Thr  
Pro205Gly + Trp208His + Tyr210His + Leu221Gln  
Tyr210Leu + Thr212Pro + Tyr218Gln + Ala219Glu  
Gly206Gln + Thr212Gln + Tyr218His + Gly223Asp  
Trp208Ile + Tyr213His + Thr215Ser + Ser222Glu  
Trp208Val + Pro214Asn + Thr215Gly + Leu221Asp  
Pro205Asn + Tyr213Met + Pro214Ser + Thr224Ser  
Pro205Gly + Trp208Met + Ile209Leu + Tyr213Met  
Pro205Gly + Thr217Glu + Tyr218Leu + Ala219His  
Trp208Val + Tyr213Gly + Ser216Asp + Leu221Thr  
Tyr210Gln + Thr212Pro + Tyr218Gly + Gly223Glu  
Pro205Gly + Trp208Asp + Ile209His + Tyr210Ala  
Trp208Ala + Ile209Leu + Tyr210Glu + Tyr213Cys  
Gly206Asn + Trp208Leu + Tyr213Val + Ser216Asp  
Ala204Pro + Pro205Gly + Thr217Glu + Leu221Ala  
Trp208Asn + Thr212Asn + Tyr213His + Ser216Glu  
Ala204His + Thr212Gly + Ala219Gln + Leu221Thr  
Gly206Gln + Thr212Pro + Tyr213Met + Thr224Pro  
Trp208Thr + Ile209Gln + Tyr213Ser + Ser222Asp  
Ala204Asn + Ile209Asn + Tyr213Pro + Leu221Pro  
Pro205Gly + Tyr210His + Tyr218Ile + Leu221Asn  
Ala204Pro + Tyr210Cys + Pro214Gln + Thr215Pro  
Pro205Ser + Tyr213Ser + Ser216Asp + Thr217Gly  
Pro205Asn + Trp208Ser + Ala219Glu + Ser220Asp  
Tyr210Ala + Tyr218Met + Ala219Glu + Ser220Asp  
Ala204Gly + Pro205Gln + Pro214Asp + Thr215Asp  
Tyr210Ser + Thr215Asn + Ser222Glu + Gly223Glu  
Gly206Pro + Tyr213Val + Ser222Glu + Gly223Asp  
Pro214Ser + Leu221Glu + Ser222Asp + Gly223Gln  
Pro205Gln + Ser216Asp + Thr217Glu + Ala219Thr

Trp208Leu + Thr212Gln + Ser220Glu + Leu221Glu  
 Gly206Gln + Ser220Asp + Leu221Asp + Gly223Asn  
 Thr217Asp + Tyr218Asp + Gly223Ser + Thr224Gly  
 Pro205Gln + Ile209Ala + Thr217Glu + Tyr218Glu  
 Pro214Gln + Thr215Asp + Ser216Glu + Thr224Pro  
 Ala204His + Ser207Glu + Tyr213Cys + Ser222Asp  
 Pro205Gly + Ser207Glu + Thr212Gln + Ser222Asp  
 Ser207Glu + Tyr210Ser + Tyr218Leu + Ser222Glu  
 Ser207Glu + Tyr218Cys + Ser222Glu + Thr224Pro  
 Pro205Ser + Trp208Glu + Leu221Glu + Ser222Glu  
 Trp208Glu + Ile209Asn + Thr217Gly + Ser222Glu  
 Ser216Glu + Thr217Asp + Tyr218Glu + Thr224Gly  
 Trp208Asp + Tyr218Leu + Ala219Thr + Leu221Glu  
 Ala204His + Thr212Gly + Thr215Glu + Thr217Glu  
 Pro214Asp + Ser216Asp + Ala219His + Gly223Asn  
 Tyr210Pro + Pro214Asp + Ser216Glu + Tyr218Ile  
 Pro205Gln + Tyr210Leu + Pro214Glu + Thr217Asp  
 Ala204Ser + Ser207Glu + Pro214Ser + Gly223Asp  
 Ser207Asp + Thr212Pro + Thr217Gly + Gly223Asp  
 Ser207Glu + Pro214Asn + Ser220Glu + Ser222Asp  
 Pro205Ser + Thr217Pro + Ala219Glu + Leu221Glu  
 Trp208Gln + Tyr210Glu + Thr212Asn + Leu221Glu  
 Trp208Gln + Thr215Pro + Ser220Glu + Ser222Glu  
 Gly206Pro + Thr217Gly + Ser220Asp + Ser222Glu  
 Gly206Gln + Tyr210Gly + Ser220Glu + Ser222Asp  
 Ser207Glu + Thr212Asn + Tyr218Pro + Leu221Asp  
 Ala204Asn + Ser207Glu + Trp208His + Leu221Asp  
 Tyr213Ile + Tyr218Leu + Ser222Asp + Thr224Glu  
 Gly206Gln + Tyr210Met + Ser222Asp + Thr224Glu  
 Ile209Ser + Tyr210His + Ser222Glu + Thr224Asp  
 Ala204Ser + Tyr218Asp + Ala219Pro + Ser220Glu  
 Gly206Gln + Thr217Ser + Tyr218Glu + Ser220Glu  
 Pro214Asp + Tyr218Asp + Gly223Ser + Thr224Gly  
 Gly206Gln + Tyr210Gly + Pro214Glu + Tyr218Glu  
 Trp208Asp + Thr217Gln + Ser220Asp + Gly223Asp  
 Ser207Glu + Tyr210Ala + Thr215Asn + Ser220Asp  
 Ser207Glu + Thr217Gly + Ser220Asp + Leu221Gln  
 Ser207Glu + Thr217Gly + Tyr218Asn + Ser220Asp  
 Pro205Asn + Ser207Asp + Thr212Gly + Ser220Asp  
 Ser207Glu + Thr212Asn + Ala219Gly + Ser220Asp  
 Gly206Pro + Ser207Glu + Trp208Asp + Thr224Asp  
 Ser207Asp + Trp208Thr + Tyr218Gln + Thr224Glu  
 Ala204Thr + Ser207Asp + Leu221Ala + Thr224Glu  
 Ser207Asp + Ala219Glu + Ser220Asp + Gly223Ser  
 Ser207Glu + Tyr210Glu + Thr215Gln + Thr224Asn  
 Ala204Gln + Tyr210Glu + Thr212Asn + Ser222Asp

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 Loop 6 - Quintuple Substitution Variants
 

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Gly206Asn + Tyr210Gly + Thr212Gln + Tyr218Ile +  
 Thr224Gly

Ala204Asn + Pro205Gly + Trp208Glu + Thr215Asn +  
Gly223Pro  
Pro205Gln + Gly206Pro + Trp208Asn + Thr212Gln +  
Thr224Ser  
Ala204Ser + Pro205Ser + Tyr213Ser + Thr215Ser +  
Ala219Gly  
Ala204Gly + Gly206Gln + Thr212Pro + Ser220Asp +  
Leu221Ser  
Pro214Ser + Thr215Gln + Tyr218Val + Ser222Asp +  
Thr224Asn  
Ala204Pro + Ser207Asp + Tyr210Cys + Thr212Pro +  
Thr215Pro  
Gly206Asn + Pro214Gln + Ala219Gly + Gly223Asn +  
Thr224Asp  
Ala204Gln + Pro205Ser + Gly206Ser + Tyr210Gln +  
Ala219Asp  
Gly206Pro + Ser207Asp + Trp208Met + Thr215Pro +  
Leu221Pro  
Ala204Asn + Pro205Gln + Tyr210Cys + Thr212Asn +  
Leu221Ser  
Ala204Asn + Gly206Gln + Trp208Thr + Ile209Ser +  
Ser222Asp  
Pro205Gln + Tyr213Leu + Thr217Glu + Tyr218Met +  
Leu221Gln  
Ala204Pro + Trp208Met + Ile209Val + Tyr210Leu +  
Ser216Glu  
Trp208Ser + Ile209Ser + Tyr218His + Ala219His +  
Gly223Pro  
Trp208Pro + Ile209Thr + Thr212Gly + Thr215Gly +  
Thr224Glu  
Ile209Val + Pro214Ser + Ser216Asp + Tyr218Met +  
Leu221Met  
Gly206Gln + Ile209Gln + Ser220Asp + Leu221Gly +  
Thr224Ser  
Pro205Gln + Trp208Cys + Thr212Asn + Tyr213Cys +  
Pro214Asp  
Pro205Gln + Trp208Gln + Thr217Asp + Gly223Ser +  
Thr224Gly  
Ser207Glu + Thr212Ser + Thr217Gly + Tyr218Gln +  
Leu221His  
Tyr210His + Thr212Ser + Leu221Glu + Gly223Ser +  
Thr224Pro  
Trp208Phe + Tyr213Met + Thr215Pro + Ser220Glu +  
Thr224Gly  
Gly206Ser + Trp208Phe + Ile209Ser + Tyr213Cys +  
Ser216Glu  
Ala204Gln + Pro205Ser + Tyr210His + Tyr218Pro +  
Thr224Glu  
Pro205Gln + Gly206Gln + Leu221His + Ser222Asp +  
Gly223Gln  
Ala204Gly + Ile209His + Tyr210Met + Ser216Glu +  
Thr217Pro  
Gly206Pro + Ile209Ala + Tyr210Gly + Leu221Asp +

Gly223Gln  
Gly206Asn + Tyr210Met + Thr215Glu + Gly223Asn +  
Thr224Gly  
Gly206Gln + Ile209Pro + Ser216Asp + Leu221Cys +  
Gly223Pro  
Trp208Thr + Tyr210Val + Thr212Ser + Pro214Asp +  
Gly223Gln  
Thr212Gly + Pro214Gln + Thr215Ser + Leu221Gly +  
Thr224Glu  
Ala204Thr + Thr212Ser + Thr217Ser + Gly223Asp +  
Thr224Pro  
Pro205Ser + Ile209Pro + Tyr213Ile + Ala219Pro +  
Ser222Glu  
Trp208Gly + Thr212Gln + Tyr218Gly + Ser222Glu +  
Gly223Ser  
Gly206Pro + Ile209Pro + Tyr213Ser + Thr217Gly +  
Tyr218Val  
Pro205Gln + Tyr213Cys + Pro214Gln + Ser220Glu +  
Thr224Gly  
Gly206Asn + Thr212Gly + Tyr213Asn + Tyr218Pro +  
Ser220Glu  
Ala204Pro + Pro205Ser + Thr212Gln + Thr217Asp +  
Thr224Ser  
Pro205Asn + Trp208Ile + Thr212Gln + Pro214Ser +  
Thr217Gly  
Ile209Cys + Thr217Gly + Ser220Asp + Leu221Gln +  
Gly223Gln  
Ile209Thr + Thr215Gly + Ser216Glu + Tyr218Leu +  
Ala219Pro  
Ile209Met + Pro214Gly + Thr217Gly + Tyr218Cys +  
Leu221Met  
Pro205Gly + Pro214Asn + Tyr218Ile + Ala219Gly +  
Ser222Asp  
Tyr210Thr + Thr212Ser + Pro214Ser + Leu221Asn +  
Ser222Asp  
Gly206Asn + Tyr210Ile + Pro214Gln + Ala219Asn +  
Leu221Val  
Pro205Asn + Thr215Ser + Ala219Ser + Leu221Ala +  
Thr224Asp  
Gly206Pro + Pro214Asn + Thr215Gln + Tyr218Ser +  
Leu221Thr  
Ala204Pro + Gly206Gln + Ile209His + Thr212Gly +  
Gly223Glu  
Pro205Gln + Tyr213Gly + Tyr218Ala + Gly223Glu +  
Thr224Pro  
Pro205Ser + Pro214Gly + Thr217Pro + Ala219Thr +  
Leu221Glu  
Ala204Pro + Pro205Asn + Ser207Asp + Tyr210Ala +  
Pro214Ser  
Gly206Ser + Tyr213Thr + Ala219Gln + Ser220Glu +  
Gly223Ser  
Pro205Gly + Thr212Asn + Thr215Pro + Ser216Glu +  
Leu221Asn



Ser207Glu + Ile209Ala + Thr212Asn + Thr215Gln +  
Tyr218Met  
Tyr213His + Thr215Gln + Thr217Asn + Ala219Glu +  
Leu221Ser  
Gly206Gln + Tyr213Asn + Thr215Ser + Tyr218Ala +  
Leu221Ala  
Trp208Met + Tyr210Met + Tyr218Gln + Ser220Glu +  
Thr224Ser  
Ala204Thr + Pro205Ser + Ile209Leu + Thr217Asn +  
Thr224Pro  
Pro205Gly + Ile209Leu + Thr215Asn + Ser216Glu +  
Gly223Ser  
Ile209Ser + Tyr213Leu + Thr217Asp + Tyr218Asn +  
Ala219His  
Ala204Ser + Pro205Ser + Ser207Glu + Leu221Val +  
Gly223Pro  
Ala204His + Thr212Gly + Ala219Gln + Leu221Thr +  
Gly223Glu  
Pro205Ser + Gly206Gln + Thr212Pro + Gly223Pro +  
Thr224Pro  
Ala204Asn + Trp208Thr + Tyr213Pro + Leu221Pro +  
Ser222Asp  
Pro205Gly + Ile209Met + Tyr218Met + Leu221Val +  
Gly223Asn  
Thr212Gly + Tyr218Ile + Ala219Asn + Ser220Glu +  
Thr224Pro  
Ala204Gln + Pro205Gln + Gly206Gln + Ile209Met +  
Ser216Asp  
Ala204Thr + Thr212Gly + Ala219Asn + Gly223Asp +  
Thr224Asn  
Ala204Pro + Trp208His + Tyr213Leu + Thr217Ser +  
Ser222Glu  
Pro205Ser + Ser207Asp + Ile209Asn + Tyr213Met +  
Thr217Gln  
Gly206Pro + Trp208Ser + Thr212Gly + Tyr213Cys +  
Gly223Glu  
Ala204Asn + Gly206Pro + Trp208Val + Pro214Asn +  
Tyr218Glu  
Ile209Gly + Tyr210Ala + Tyr218Pro + Gly223Ser +  
Thr224Ser  
Pro205Gln + Tyr213His + Tyr218Met + Ser220Glu +  
Leu221Gly  
Ala204Asn + Tyr210Cys + Thr212Gln + Tyr218Leu +  
Ser222Asp  
Gly206Ser + Ile209Leu + Thr212Gly + Pro214Gln +  
Thr215Glu  
Ser207Asp + Pro214Ser + Thr215Asn + Thr217Asn +  
Leu221Ser  
Pro205Gly + Trp208Thr + Thr217Gly + Ser220Glu +  
Gly223Ser  
Gly206Ser + Trp208Glu + Tyr210Gln + Thr217Gln +  
Leu221Pro  
Gly206Gln + Ser207Glu + Thr212Asn + Pro214Ser +

Leu221Ala  
Ala204Ser + Trp208Leu + Ile209Asn + Thr215Asp +  
Thr224Asn  
Gly206Ser + Ile209Pro + Thr217Ser + Ser220Glu +  
Gly223Gln  
Pro205Gln + Trp208Pro + Thr212Pro + Pro214Glu +  
Ala219Ser  
Gly206Pro + Ser207Glu + Tyr210Met + Thr212Gly +  
Tyr213Leu  
Ala204His + Tyr210Pro + Tyr213Ala + Thr217Glu +  
Thr224Ser  
Trp208Cys + Ile209Met + Tyr210Ala + Tyr213Met +  
Ala219Asn  
Trp208Thr + Tyr210Ala + Tyr218Met + Ala219Glu +  
Ser220Asp  
Trp208Asn + Ile209Pro + Ala219Asp + Ser220Asp +  
Gly223Ser  
Pro205Ser + Tyr213Thr + Pro214Asp + Thr215Asp +  
Tyr218Val  
Ile209Ser + Tyr213Pro + Leu221Met + Ser222Asp +  
Gly223Asp  
Ala204Thr + Thr217Gln + Tyr218Ile + Ser222Glu +  
Gly223Asp  
Pro205Ser + Tyr210Ala + Thr215Asn + Ser222Glu +  
Gly223Glu  
Tyr210Pro + Thr212Pro + Leu221Asn + Ser222Asp +  
Gly223Asp  
Trp208Pro + Ile209Pro + Tyr213Ile + Ser222Asp +  
Gly223Asp  
Gly206Gln + Tyr213Gly + Thr217Gly + Ser222Glu +  
Gly223Glu  
Trp208Ser + Ile209Pro + Thr212Pro + Ser216Glu +  
Thr217Asp  
Gly206Asn + Tyr218Met + Ala219His + Ser220Glu +  
Leu221Asp  
Ile209Ala + Thr215Glu + Ser216Glu + Gly223Gln +  
Thr224Asn  
Ile209Gly + Pro214Ser + Thr215Glu + Ser216Asp +  
Tyr218Ile  
Ala204Ser + Trp208Val + Ile209Ser + Thr215Glu +  
Ser216Asp  
Thr212Pro + Thr215Asp + Ser216Glu + Thr217Glu +  
Thr224Ser  
Ala204His + Ser207Glu + Trp208His + Tyr210His +  
Ser222Asp  
Ser207Glu + Tyr213Asn + Leu221Thr + Ser222Asp +  
Thr224Gly  
Gly206Ser + Ser207Glu + Trp208Pro + Ala219Thr +  
Ser222Asp  
Pro205Gly + Ser207Glu + Thr212Gln + Tyr213Gln +  
Ser222Asp  
Pro205Ser + Ser207Glu + Tyr213His + Thr217Pro +  
Ser222Glu

Gly206Pro + Ser207Asp + Tyr213Ile + Tyr218Asn +  
Ser222Glu  
Gly206Pro + Trp208Glu + Tyr210Cys + Pro214Gln +  
Ser220Asp  
Tyr210Glu + Tyr213Gly + Tyr218Leu + Ala219Asp +  
Thr224Ser  
Gly206Pro + Ser207Asp + Leu221Asp + Ser222Glu +  
Thr224Gln  
Tyr213Ser + Pro214Ser + Thr215Glu + Thr217Asp +  
Tyr218Ala  
Ala204Asn + Tyr210Gln + Thr212Asn + Pro214Glu +  
Ser216Asp  
Pro205Gln + Gly206Pro + Pro214Glu + Ser216Asp +  
Thr224Ser  
Ser207Asp + Thr212Pro + Thr215Gly + Thr217Gly +  
Gly223Asp  
Ser207Glu + Tyr210Val + Pro214Asn + Leu221Thr +  
Gly223Glu  
Ser207Asp + Ile209Thr + Tyr210Met + Thr212Pro +  
Gly223Asp  
Ser207Glu + Tyr210Ser + Tyr218Leu + Ser220Asp +  
Ser222Glu  
Ser207Asp + Thr217Gln + Ser220Asp + Ser222Asp +  
Thr224Asn  
Trp208Thr + Thr217Glu + Tyr218Ser + Ala219Glu +  
Leu221Cys  
Pro205Gly + Thr212Ser + Ala219Gln + Ser220Glu +  
Ser222Glu  
Gly206Pro + Trp208His + Tyr210Gly + Ser220Glu +  
Ser222Asp  
Ala204Gln + Gly206Ser + Trp208Gln + Ser220Glu +  
Ser222Glu  
Ala204Ser + Tyr213Thr + Ser220Asp + Leu221Met +  
Ser222Asp  
Thr212Gly + Thr215Asp + Ser216Asp + Thr217Gly +  
Tyr218Asp  
Ala204Asn + Tyr210Glu + Tyr213Ala + Ser220Glu +  
Ser222Glu  
Ala204His + Gly206Pro + Ser216Glu + Tyr218Asp +  
Leu221Ser  
Gly206Pro + Ile209Ser + Tyr210His + Ser222Glu +  
Thr224Asp  
Trp208Met + Tyr218Leu + Ala219Pro + Ser222Glu +  
Thr224Glu  
Trp208Val + Tyr210Leu + Thr212Asn + Tyr218Asp +  
Ser220Glu  
Ala204Ser + Tyr213Gln + Tyr218Asp + Ala219Pro +  
Ser220Glu  
Thr212Asn + Tyr213Gln + Thr217Ser + Tyr218Glu +  
Ser220Glu  
Pro214Ser + Leu221Glu + Ser222Asp + Gly223Ser +  
Thr224Asp  
Pro205Gly + Tyr210Cys + Ala219Asp + Ser220Glu +

Ser222Asp  
Gly206Gln + Tyr210Gly + Pro214Glu + Thr217Pro +  
Tyr218Glu  
Trp208Asp + Pro214Ser + Thr217Gln + Ser220Asp +  
Gly223Asp  
Ser207Glu + Tyr210Ala + Thr215Asn + Ser220Asp +  
Leu221Ala  
Ser207Asp + Trp208Cys + Ile209Ala + Thr215Asn +  
Ser220Glu  
Ser207Asp + Tyr210Met + Tyr218Asn + Ser220Glu +  
Leu221Cys  
Ser207Glu + Tyr210Pro + Thr215Gln + Tyr218Cys +  
Ser220Glu  
Ser207Glu + Thr212Ser + Pro214Gln + Ser220Asp +  
Leu221Pro  
Pro205Asn + Ser207Asp + Thr215Pro + Ser220Asp +  
Leu221Thr  
Trp208Glu + Ile209Asn + Thr217Gly + Ala219Asp +  
Ser222Glu  
Pro205Asn + Ser207Glu + Tyr213Leu + Ser220Asp +  
Gly223Glu  
Ala204Ser + Pro205Gly + Ser207Asp + Thr212Ser +  
Thr224Asp  
Ala204Pro + Gly206Gln + Ser207Glu + Tyr218Asn +  
Thr224Glu  
Gly206Ser + Ser207Glu + Tyr210Met + Tyr213Cys +  
Thr224Asp  
Pro205Asn + Ser207Glu + Ile209Val + Tyr213Pro +  
Thr224Glu  
Ser207Asp + Ile209Val + Tyr213Ala + Gly223Gln +  
Thr224Glu  
Pro205Asn + Ser207Glu + Ala219Glu + Ser220Glu +  
Gly223Gln  
Gly206Asn + Ser207Glu + Trp208Asp + Ala219Asp +  
Thr224Pro  
Ala204Gly + Gly206Ser + Ser207Asp + Tyr210Asp +  
Leu221Gly  
Ala204Pro + Ser207Glu + Ile209Thr + Tyr210Glu +  
Tyr218Leu  
Ile209Thr + Tyr213Gly + Ala219Asp + Ser222Glu +  
Gly223Asp  
Pro205Gln + Tyr213Gln + Tyr218Glu + Leu221Glu +  
Ser222Asp  
Gly206Gln + Ser207Glu + Thr212Asn + Ser220Glu +  
Thr224Glu  
Ser207Glu + Thr217Gly + Tyr218Asn + Ser220Asp +  
Thr224Glu  
Ala204Thr + Tyr210Cys + Ser220Glu + Leu221Ser +  
Gly223Glu  
Ile209Pro + Ser216Glu + Ala219Glu + Ser220Asp +  
Gly223Asn  
Gly206Gln + Tyr210Met + Thr212Pro + Ala219Glu +  
Ser222Asp

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Pro205Asn + Tyr210Glu + Thr217Asp + Tyr218Ala + Thr224Pro  
Ala204Gly + Trp208Gln + Thr212Gln + Ser216Asp + Ala219Glu  
Ala204Asn + Trp208Ala + Ser216Asp + Ala219Glu + Thr224Gly  
Ala204Asn + Tyr210Asp + Thr215Glu + Thr217Glu + Ala219Gly  
Trp208Leu + Ile209Gly + Thr215Asp + Thr217Glu + Ser220Asp  
Thr212Pro + Tyr213Leu + Ser216Glu + Tyr218Glu + Leu221Glu  
Thr217Asp + Ala219Glu + Leu221Asn + Ser222Glu + Thr224Ser  
Pro205Ser + Trp208Glu + Tyr218Glu + Leu221Gly + Ser222Glu  
Ser207Glu + Thr215Pro + Thr217Asp + Ser220Glu + Gly223Pro  
Thr217Asp + Ala219Asn + Leu221Glu + Ser222Glu + Gly223Asn  
Trp208Tyr + Ile209Asn + Thr217Glu + Leu221Glu + Ser222Asp  
Ala204Gly + Tyr210Glu + Pro214Glu + Ser216Glu + Thr217Asn  
Pro214Asp + Thr215Gln + Ser216Glu + Ser220Glu + Leu221Met  
Ala204Gln + Tyr210Glu + Thr212Asn + Thr217Glu + Ser222Asp  
Trp208Leu + Ile209Val + Thr215Asp + Thr217Glu + Leu221Glu  
Gly206Pro + Ile209Val + Tyr213Pro + Thr215Asp + Ala219Glu  
Pro205Asn + Ser207Glu + Tyr218Asp + Ser222Asp + Thr224Gly  
Gly206Ser + Tyr210Asp + Pro214Asp + Thr215Pro + Leu221Asp  
Tyr210Ser + Pro214Asp + Tyr218Cys + Ser220Glu + Ser222Glu  
Ser207Asp + Ile209Ser + Thr212Ser + Pro214Asp + Thr215Asp

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### Loop 6 - Sextuple Substitution Variants

Gly206Asn + Tyr210Gly + Thr212Gln + Tyr213Gly +  
Tyr218Ile + Thr224Gly  
Pro205Asn + Gly206Ser + Tyr213Gly + Ser216Asp +  
Thr217Ser + Thr224Asn  
Ala204Ser + Pro205Ser + Tyr213Ser + Tyr218His +  
Ala219Gln + Leu221Asn  
Ser207Glu + Thr212Gln + Thr215Pro + Tyr218Gln +  
Leu221Met + Gly223Gln

Pro205Ser + Ser207Asp + Tyr210Val + Tyr213Leu +  
Leu221Gln + Thr224Gly  
Ala204Ser + Trp208Cys + Ile209His + Thr215Glu +  
Thr217Pro + Gly223Ser  
Ala204Gly + Ile209Asn + Tyr210Ile + Ser216Asp +  
Ala219Asn + Leu221Met  
Ala204Gly + Pro205Gln + Pro214Glu + Thr215Ser +  
Tyr218Asn + Thr224Ser  
Gly206Asn + Trp208Cys + Thr212Gly + Tyr213Gly +  
Ser216Asp + Leu221His  
Trp208Pro + Ile209Gly + Thr215Ser + Ala219Thr +  
Leu221Thr + Thr224Asp  
Pro205Gly + Gly206Gln + Ile209Gly + Thr212Asn +  
Pro214Asn + Ser220Glu  
Pro205Gly + Trp208Leu + Thr217Gln + Leu221Gln +  
Gly223Gln + Thr224Glu  
Ala204Gly + Gly206Asn + Tyr210Cys + Tyr213Thr +  
Gly223Asp + Thr224Asn  
Gly206Asn + Trp208His + Ile209Gly + Thr217Gly +  
Leu221Val + Thr224Pro  
Ala204Thr + Thr212Gln + Tyr213Gln + Thr217Ser +  
Ser222Asp + Thr224Pro  
Trp208Gly + Thr212Gln + Tyr213Leu + Tyr218Gly +  
Ser222Glu + Gly223Ser  
Pro205Ser + Trp208Ser + Tyr213Asn + Tyr218Gln +  
Ser222Asp + Thr224Gln  
Ala204His + Pro205Gly + Ile209Asn + Tyr213Gln +  
Thr215Ser + Thr217Ser  
Ala204Thr + Gly206Asn + Trp208Leu + Thr215Ser +  
Ser216Asp + Ala219His  
Gly206Ser + Thr212Gln + Tyr213Val + Tyr218Cys +  
Ser220Glu + Thr224Gln  
Gly206Asn + Trp208Ile + Ile209Ser + Tyr218Gln +  
Gly223Glu + Thr224Gly  
Gly206Asn + Ser207Glu + Ile209Cys + Thr212Ser +  
Thr217Pro + Ala219His  
Ala204Asn + Tyr210Cys + Tyr213Asn + Tyr218Leu +  
Leu221His + Ser222Glu  
Gly206Pro + Tyr213Asn + Pro214Asn + Thr215Gln +  
Tyr218Ser + Leu221Thr  
Ala204Pro + Pro205Asn + Gly206Gln + Ile209His +  
Thr212Gly + Gly223Glu  
Pro205Gln + Tyr213Gly + Thr215Gly + Tyr218Ala +  
Gly223Glu + Thr224Pro  
Ala204His + Ser207Glu + Trp208Gly + Ile209His +  
Tyr213Cys + Thr215Ser  
Gly206Ser + Trp208His + Tyr213Thr + Ala219Gln +  
Ser220Glu + Gly223Ser  
Tyr213His + Thr215Gln + Thr217Asn + Tyr218Ala +  
Ala219Glu + Leu221Ser  
Ala204Asn + Pro205Ser + Gly206Asn + Ile209Leu +  
Thr215Asp + Thr224Pro  
Pro205Ser + Ile209Gly + Tyr213Leu + Thr217Asp +

Tyr218Asn + Ala219His  
Ala204Asn + Pro205Gln + Ser207Asp + Tyr210Val +  
Thr212Gly + Leu221Thr  
Ala204Asn + Trp208Thr + Ile209Gln + Tyr213Pro +  
Leu221Pro + Ser222Asp  
Ala204Ser + Gly206Ser + Tyr213Gly + Pro214Gln +  
Tyr218Val + Ser222Asp  
Ala204Pro + Tyr210Cys + Tyr213Ser + Pro214Gln +  
Thr215Pro + Thr217Gly  
Thr212Ser + Pro214Asp + Thr217Gly + Ala219Thr +  
Leu221Pro + Thr224Gln  
Pro205Gln + Gly206Gln + Ser207Glu + Tyr210Gly +  
Thr212Pro + Tyr218Gly  
Pro205Gln + Gly206Pro + Trp208Asn + Ile209Met +  
Tyr213Ser + Ala219Asp  
Ala204Asn + Ser207Glu + Trp208Ala + Tyr213Asn +  
Leu221Thr + Thr224Gly  
Ala204Pro + Trp208His + Ile209Asn + Tyr213Leu +  
Thr217Ser + Ser222Glu  
Gly206Pro + Trp208Ser + Tyr210Val + Thr212Gly +  
Tyr213Cys + Ala219Asp  
Pro205Gly + Gly206Gln + Ile209Cys + Tyr210His +  
Thr215Gln + Ala219Thr  
Trp208Gly + Ile209Ser + Thr212Gln + Tyr218Met +  
Leu221Ser + Gly223Ser  
Pro205Asn + Ile209Asn + Thr212Asn + Thr217Glu +  
Gly223Pro + Thr224Ser  
Pro205Gly + Trp208Thr + Thr212Ser + Ser216Glu +  
Thr217Gly + Gly223Ser  
Gly206Ser + Trp208Glu + Tyr210Gln + Thr215Gly +  
Thr217Gln + Leu221Pro  
Gly206Ser + Ser207Glu + Trp208Leu + Thr212Asn +  
Leu221Ala + Thr224Asn  
Ala204Asn + Pro205Gln + Gly206Gln + Trp208Ile +  
Ser216Asp + Tyr218Cys  
Ala204Thr + Tyr210Cys + Tyr213Ser + Thr215Ser +  
Ser216Asp + Ala219His  
Trp208Leu + Ile209Asn + Thr212Gly + Thr215Ser +  
Tyr218Pro + Leu221Gly  
Pro205Gly + Gly206Gln + Tyr218Gly + Ala219Asn +  
Leu221Gln + Thr224Ser  
Pro205Gln + Trp208Val + Tyr210Pro + Thr215Gly +  
Ala219Ser + Thr224Glu  
Pro205Gly + Trp208Met + Ile209Ser + Thr212Asn +  
Tyr218Ala + Ala219Thr  
Thr212Gly + Tyr213Asn + Pro214Ser + Ser216Glu +  
Thr217Pro + Leu221Thr  
Ala204Gly + Trp208Pro + Ile209Pro + Tyr213Gln +  
Tyr218Ser + Ser220Glu  
Ala204Gly + Gly206Asn + Ile209Met + Thr212Pro +  
Ala219Glu + Leu221Met  
Pro205Asn + Gly206Pro + Trp208Leu + Tyr210Pro +  
Thr217Gln + Tyr218Glu

Pro205Asn + Gly206Gln + Tyr213Val + Ser220Asp +  
Gly223Ser + Thr224Asn  
Ala204Thr + Pro205Gly + Gly206Gln + Pro214Gln +  
Leu221Val + Gly223Gln  
Pro205Ser + Ile209Gln + Thr212Pro + Ala219Gly +  
Ser220Glu + Gly223Gln  
Ala204Gly + Gly206Gln + Ile209Leu + Thr212Asn +  
Pro214Gln + Thr215Ser  
Gly206Asn + Trp208Ser + Thr212Ser + Pro214Asn +  
Ser220Glu + Gly223Ser  
Ala204His + Tyr213Leu + Pro214Asn + Thr217Pro +  
Ser220Glu + Gly223Gln  
Gly206Pro + Ile209Cys + Pro214Gln + Thr217Ser +  
Tyr218Cys + Ala219Gln  
Ala204Thr + Tyr210Ala + Thr215Gly + Thr217Gln +  
Ala219Pro + Ser220Asp  
Ser207Asp + Ile209Thr + Thr212Gly + Thr217Pro +  
Ala219Gly + Thr224Pro  
Ala204Gly + Ser207Glu + Thr212Asn + Pro214Ser +  
Tyr218Ser + Ala219Ser  
Pro205Gln + Gly206Gln + Thr212Ser + Tyr213Ala +  
Thr215Ser + Gly223Glu  
Pro205Ser + Tyr210Ala + Pro214Ser + Thr215Asn +  
Ser222Asp + Gly223Glu  
Ile209Pro + Tyr210Pro + Thr212Pro + Leu221Asn +  
Ser222Asp + Gly223Asp  
Pro205Ser + Thr212Ser + Tyr213Ile + Ala219Pro +  
Ser222Glu + Gly223Asp  
Ala204Asn + Ile209Cys + Tyr213Cys + Tyr218Ala +  
Leu221Asp + Ser222Glu  
Ile209Val + Thr212Asn + Tyr213Met + Thr217Asn +  
Gly223Asp + Thr224Asp  
Pro205Gly + Ile209Asn + Thr212Asn + Ser216Glu +  
Thr217Glu + Tyr218Gln  
Ala204His + Trp208Ser + Thr212Pro + Tyr213Pro +  
Ser216Glu + Thr217Asp  
Pro205Ser + Ile209Ala + Thr212Gly + Ser216Asp +  
Thr217Glu + Ala219Thr  
Pro205Ser + Trp208Met + Pro214Asn + Ala219Gln +  
Ser220Glu + Leu221Asp  
Ala204Pro + Gly206Ser + Tyr213Ile + Ser220Glu +  
Leu221Asp + Gly223Ser  
Gly206Pro + Tyr210Gly + Tyr213His + Thr217Glu +  
Tyr218Asp + Leu221Asn  
Thr212Pro + Tyr213Ser + Thr215Asp + Ser216Asp +  
Tyr218Pro + Gly223Gln  
Ser207Glu + Ile209Asn + Thr212Ser + Thr217Gly +  
Ser222Glu + Thr224Gln  
Pro205Gly + Ser207Glu + Tyr210Ser + Pro214Gln +  
Ser222Asp + Gly223Asn  
Gly206Pro + Ser207Asp + Trp208Ser + Pro214Ser +  
Leu221Asn + Ser222Asp  
Gly206Gln + Ser207Asp + Ile209Cys + Tyr210Met +



Thr217Asn + Ser222Glu  
Gly206Pro + Ser207Glu + Ile209Pro + Tyr210Ile +  
Leu221Asn + Ser222Glu  
Pro205Ser + Ser207Glu + Trp208His + Thr215Pro +  
Leu221Asn + Ser222Glu  
Pro205Asn + Ser207Asp + Thr215Gln + Tyr218Ile +  
Leu221Gly + Ser222Glu  
Ala204Pro + Ser207Asp + Tyr210Cys + Thr212Pro +  
Thr215Pro + Ser222Glu  
Pro205Gly + Pro214Asp + Thr215Glu + Ser216Asp +  
Thr217Pro + Gly223Gln  
Ile209Thr + Thr217Gly + Ala219Glu + Ser220Asp +  
Leu221Glu + Gly223Ser  
Ala204Gln + Ser207Asp + Ile209Thr + Tyr218Asn +  
Ser222Glu + Gly223Asp  
Pro205Ser + Ser207Glu + Pro214Gly + Thr215Asn +  
Ser222Asp + Gly223Glu  
Pro205Asn + Gly206Pro + Trp208Asp + Thr212Gln +  
Ser222Asp + Gly223Ser  
Gly206Gln + Pro214Asp + Thr215Asp + Thr217Asp +  
Gly223Ser + Thr224Gly  
Ala204His + Ile209Thr + Ser216Asp + Thr217Asp +  
Tyr218Asp + Leu221Ser  
Thr212Asn + Pro214Gln + Tyr218Met + Ser222Glu +  
Gly223Asp + Thr224Glu  
Ala204Gln + Gly206Pro + Tyr210Glu + Pro214Gly +  
Ala219Asp + Leu221Gln  
Ala204Gly + Pro205Gln + Trp208Asp + Ile209Gln +  
Pro214Gly + Leu221Glu  
Ala204Asn + Gly206Gln + Ser207Glu + Trp208His +  
Leu221Asp + Ser222Asp  
Trp208Gly + Tyr213Ser + Pro214Ser + Thr215Glu +  
Thr217Asp + Tyr218Ala  
Ala204Asn + Pro205Ser + Tyr210Pro + Pro214Asp +  
Ser216Glu + Tyr218Ile  
Gly206Gln + Ile209Leu + Thr212Gly + Pro214Asp +  
Ser216Glu + Leu221His  
Pro205Gln + Trp208Glu + Tyr210Asp + Thr212Gln +  
Tyr213Asn + Thr215Ser  
Ser207Asp + Thr212Pro + Thr215Gly + Thr217Gln +  
Leu221Met + Gly223Asp  
Ala204Asn + Pro205Gln + Ser207Glu + Trp208Met +  
Ala219Pro + Gly223Asp  
Ala204Thr + Ser207Asp + Thr212Gly + Ala219Asn +  
Gly223Asp + Thr224Asn  
Ser207Asp + Pro214Asn + Thr215Gly + Tyr218Ala +  
Leu221Ser + Gly223Asp  
Ser207Glu + Thr212Asn + Tyr213Gln + Tyr218Pro +  
Ser220Glu + Leu221Asp  
Ala204Asn + Gly206Ser + Ser207Asp + Tyr210Thr +  
Ser220Asp + Leu221Asp  
Gly206Ser + Thr212Asn + Thr215Pro + Tyr218Val +  
Leu221Asp + Gly223Asp

Trp208Met + Tyr210Thr + Thr212Asn + Ala219Gln +  
Leu221Asp + Gly223Asp  
Gly206Ser + Ser207Glu + Pro214Ser + Ala219Asn +  
Ser220Glu + Ser222Glu  
Ala204His + Ser207Glu + Ala219Ser + Ser220Glu +  
Leu221His + Ser222Asp  
Pro205Ser + Ser207Glu + Tyr213His + Thr217Pro +  
Ser220Glu + Ser222Glu  
Ser207Glu + Ile209Met + Tyr210Gly + Tyr213Leu +  
Ser220Glu + Ser222Asp  
Ser207Asp + Tyr213Pro + Pro214Gln + Ser220Glu +  
Ser222Glu + Gly223Pro  
Gly206Ser + Trp208Gln + Tyr210Glu + Thr212Asn +  
Thr215Gly + Leu221Glu  
Pro205Gly + Thr212Ser + Tyr218Leu + Ala219Gln +  
Ser220Glu + Ser222Glu  
Trp208Phe + Thr212Asn + Pro214Ser + Ser220Glu +  
Ser222Asp + Gly223Gln  
Ala204Gly + Gly206Pro + Tyr210Ser + Tyr218Cys +  
Ser220Glu + Ser222Asp  
Pro205Ser + Trp208Leu + Tyr213Ala + Thr217Ser +  
Ser220Asp + Ser222Asp  
Pro205Gly + Trp208His + Tyr210His + Ser220Glu +  
Leu221Gln + Ser222Asp  
Pro205Gly + Thr212Gln + Ala219Thr + Ser220Glu +  
Ser222Asp + Gly223Pro  
Ala204Ser + Gly206Pro + Tyr210Gly + Ser220Glu +  
Leu221Ser + Ser222Asp  
Ile209Ser + Tyr210Ser + Thr212Asn + Tyr213Cys +  
Ser220Asp + Ser222Glu  
Ala204Asn + Pro205Gly + Ser207Asp + Ile209Ser +  
Tyr213Val + Leu221Glu  
Ala204Asn + Gly206Pro + Thr215Glu + Ser216Glu +  
Thr217Asn + Tyr218Glu  
Gly206Pro + Ile209Ser + Tyr210His + Thr217Gly +  
Ser222Glu + Thr224Asp  
Gly206Gln + Thr217Gly + Ser220Asp + Ser222Glu +  
Gly223Glu + Thr224Ser  
Gly206Gln + Trp208Gln + Ile209Gln + Ser216Asp +  
Tyr218Asp + Ala219Glu  
Gly206Asn + Thr212Pro + Pro214Ser + Thr217Glu +  
Tyr218Glu + Ser220Glu  
Gly206Gln + Trp208Ser + Tyr210Asp + Pro214Gln +  
Thr217Glu + Ala219Asp  
Gly206Ser + Ser207Glu + Trp208Pro + Tyr210Asp +  
Ala219Thr + Ser222Asp  
Trp208Gly + Tyr210His + Thr212Ser + Pro214Glu +  
Tyr218Asp + Leu221Ile  
Ala204Gln + Gly206Gln + Tyr210Gly + Pro214Glu +  
Thr217Pro + Tyr218Glu  
Ala204His + Pro214Glu + Thr215Gln + Thr217Gln +  
Tyr218Glu + Leu221Pro  
Ser207Asp + Ile209Cys + Thr215Pro + Tyr218His +

Ala219Thr + Ser220Glu  
Pro205Asn + Ser207Glu + Trp208Tyr + Ile209Asn +  
Thr217Gln + Ser220Glu  
Ser207Asp + Pro214Ser + Thr215Asn + Thr217Asn +  
Ser220Glu + Leu221Ser  
Pro205Gly + Ser207Asp + Tyr210Ser + Ser220Asp +  
Gly223Pro + Thr224Ser  
Gly206Gln + Ser207Glu + Ile209Gln + Ser220Asp +  
Leu221Ala + Thr224Ser  
Ala204Ser + Pro205Gly + Ser207Asp + Thr212Ser +  
Thr215Gln + Ser220Glu  
Pro205Ser + Ser207Asp + Thr217Gln + Ala219His +  
Ser220Glu + Gly223Glu  
Ser207Glu + Thr215Gln + Tyr218Val + Ser220Asp +  
Gly223Glu + Thr224Asn  
Pro205Gly + Ile209Pro + Tyr210Asp + Ala219Asp +  
Ser222Asp + Gly223Ser  
Gly206Asn + Tyr210Gln + Pro214Asn + Thr215Glu +  
Tyr218Glu + Leu221Ala  
Ile209Asn + Tyr210Asp + Thr212Gln + Ser222Glu +  
Gly223Pro + Thr224Asn  
Ala204Gly + Gly206Gln + Ser220Asp + Leu221Asp +  
Gly223Asn + Thr224Glu  
Gly206Pro + Thr212Pro + Thr217Pro + Tyr218Asn +  
Leu221Glu + Thr224Glu  
Ile209His + Tyr210Leu + Tyr213Leu + Pro214Asp +  
Thr217Asp + Ser220Glu  
Ile209Asn + Tyr210Ile + Thr212Asn + Thr215Gly +  
Ser220Asp + Gly223Glu  
Gly206Gln + Tyr210Ala + Thr215Asn + Ser216Glu +  
Thr217Asp + Ser220Asp  
Trp208Thr + Tyr213Pro + Tyr218His + Ala219Glu +  
Leu221Cys + Ser222Asp  
Ala204Gly + Trp208Gln + Thr212Gln + Ser216Asp +  
Ala219Glu + Gly223Ser  
Gly206Ser + Pro214Gly + Ser216Asp + Tyr218Pro +  
Ala219Glu + Leu221Pro  
Pro205Asn + Tyr210Asn + Thr217Glu + Ala219Gln +  
Ser220Glu + Gly223Gln  
Ala204Thr + Tyr213Ala + Pro214Gln + Thr215Gln +  
Thr217Asp + Ser220Glu  
Trp208Gln + Tyr210His + Thr217Asp + Tyr218Met +  
Ser220Asp + Thr224Ser  
Ala204Asn + Gly206Pro + Tyr210Asp + Pro214Glu +  
Ser220Glu + Thr224Asn  
Tyr213Ile + Ser216Glu + Tyr218His + Ala219Glu +  
Leu221Asp + Thr224Ser  
Pro205Ser + Trp208Glu + Tyr218Glu + Leu221Gly +  
Ser222Glu + Thr224Gly  
Ser207Asp + Tyr213Met + Pro214Gly + Thr217Gln +  
Tyr218Asp + Ala219Glu  
Trp208Phe + Ala219Asp + Leu221Ala + Ser222Asp +  
Gly223Asn + Thr224Glu

Gly206Pro + Ile209His + Pro214Glu + Ser220Asp +  
 Leu221Glu + Thr224Pro  
 Trp208Tyr + Ile209Asn + Thr212Asn + Thr217Glu +  
 Leu221Glu + Ser222Asp  
 Pro205Gly + Ser216Glu + Thr217Glu + Ala219Gln +  
 Leu221Glu + Gly223Gln  
 Pro214Asn + Thr217Pro + Ala219Asp + Ser220Asp +  
 Leu221Gln + Thr224Asp  
 Ala204His + Gly206Asn + Ile209His + Pro214Glu +  
 Ser216Asp + Ser220Glu  
 Ser207Asp + Tyr210Glu + Thr217Asp + Tyr218Ala +  
 Leu221Ile + Thr224Pro  
 Thr212Gly + Tyr213Ala + Pro214Glu + Leu221Glu +  
 Ser222Asp + Thr224Ser  
 Pro205Gln + Gly206Asn + Thr212Gln + Tyr218Glu +  
 Ser222Asp + Gly223Asp  
 Pro205Gln + Thr212Gly + Tyr213Ala + Thr217Glu +  
 Tyr218Glu + Ser222Glu  
 Ile209Gln + Tyr213Ala + Pro214Glu + Ala219Asp +  
 Ser222Asp + Thr224Pro  
 Ala204Gly + Tyr210Pro + Pro214Glu + Ala219Glu +  
 Leu221Asn + Ser222Asp  
 Gly206Pro + Thr212Asn + Tyr213Thr + Pro214Asp +  
 Ser220Asp + Ser222Glu  
 Ala204Thr + Tyr213Ser + Pro214Asp + Ala219Asn +  
 Ser220Asp + Ser222Glu  
 Ala204His + Gly206Asn + Tyr210Pro + Pro214Glu +  
 Ser220Glu + Ser222Glu  
 Ser207Asp + Trp208Met + Pro214Glu + Thr215Asp +  
 Thr217Ser + Gly223Pro  
 Trp208Cys + Ile209Ser + Pro214Glu + Ser222Asp +  
 Gly223Asp + Thr224Gly  
 Pro205Asn + Thr212Asn + Tyr213Leu + Thr215Asp +  
 Ser222Asp + Gly223Glu

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 Loop 6 - Heptuple Substitution Mutation Variants
 

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Gly206Asn + Tyr210Gly + Thr212Gln + Thr215Gln +  
 Tyr218Ile + Leu221Ile + Thr224Gly  
 Ala204Ser + Pro205Ser + Ile209Met + Tyr213Ser +  
 Tyr218His + Ala219Gln + Leu221Asn  
 Ala204Gly + Trp208Gln + Thr212Gln + Ser216Asp +  
 Tyr218Gln + Leu221Met + Gly223Ser  
 Gly206Gln + Ile209Leu + Tyr210Ala + Thr212Gly +  
 Tyr213Gly + Ser216Asp + Gly223Gln  
 Ala204Gln + Pro205Asn + Gly206Asn + Trp208Ile +  
 Tyr210Gln + Thr215Gln + Ala219Asp  
 Ala204Asn + Pro205Gln + Ile209Ala + Tyr213Leu +  
 Thr217Glu + Tyr218Met + Leu221Gln  
 Pro205Gly + Ile209Gln + Thr212Ser + Ala219Gln +  
 Ser220Glu + Leu221Cys + Thr224Ser

Gly206Asn + Trp208Cys + Thr212Gly + Tyr213Gly +  
Ser216Asp + Thr217Gly + Leu221His  
Ala204Gln + Pro205Ser + Tyr210His + Thr212Pro +  
Tyr218Pro + Ala219Ser + Thr224Glu  
Ala204Gly + Gly206Asn + Tyr210Cys + Tyr213Thr +  
Thr215Gly + Gly223Asp + Thr224Asn  
Ala204Gln + Pro205Ser + Gly206Gln + Thr212Asn +  
Tyr218Cys + Ala219His + Gly223Glu  
Ala204Ser + Gly206Asn + Ile209Gly + Pro214Gly +  
Thr217Gly + Leu221Val + Thr224Pro  
Pro205Gly + Ile209Gln + Pro214Asn + Tyr218Ile +  
Ala219Gly + Leu221Met + Ser222Asp  
Ala204Asn + Trp208Ala + Ile209Gln + Pro214Gly +  
Thr217Glu + Tyr218Leu + Gly223Asn  
Gly206Asn + Tyr210Glu + Tyr213Gly + Thr217Gln +  
Tyr218Leu + Ala219Thr + Thr224Ser  
Gly206Pro + Ile209His + Tyr213Asn + Pro214Asn +  
Thr215Gln + Tyr218Ser + Leu221Thr  
Pro205Gly + Gly206Ser + Trp208His + Tyr213Thr +  
Ala219Gln + Ser220Glu + Gly223Ser  
Pro205Gln + Gly206Ser + Trp208Val + Tyr210Leu +  
Thr212Asn + Tyr218Met + Ser220Glu  
Ala204His + Gly206Asn + Tyr210Ser + Pro214Ser +  
Thr217Gln + Tyr218His + Ser222Asp  
Gly206Gln + Tyr213Asn + Thr215Gly + Ser216Glu +  
Thr217Gly + Tyr218His + Gly223Asn  
Gly206Gln + Ser207Glu + Tyr210Gly + Thr212Pro +  
Tyr213Met + Gly223Pro + Thr224Pro  
Ala204Asn + Trp208Thr + Ile209Gln + Tyr213Pro +  
Thr217Ser + Leu221Pro + Ser222Asp  
Ala204Ser + Gly206Gln + Trp208Asn + Thr212Ser +  
Ala219Gln + Ser220Glu + Leu221Asn  
Pro205Gly + Gly206Gln + Ile209Asn + Tyr213Val +  
Thr215Gln + Thr217Ser + Ala219Thr  
Ala204Ser + Pro205Gln + Ile209Asn + Thr212Asn +  
Thr217Glu + Gly223Pro + Thr224Ser  
Thr212Gly + Tyr213Ile + Pro214Gly + Tyr218Gly +  
Ala219Pro + Leu221Asn + Thr224Glu  
Ala204Asn + Tyr210Asp + Thr212Pro + Tyr213Asn +  
Thr217Asn + Tyr218Cys + Leu221His  
Ala204Gln + Ile209Cys + Tyr210Gln + Thr212Gly +  
Tyr213Gln + Gly223Pro + Thr224Asp  
Pro205Gln + Gly206Gln + Trp208Thr + Tyr210Leu +  
Thr215Gly + Thr217Gly + Tyr218Leu  
Tyr210Ala + Tyr213Val + Pro214Gln + Ala219Ser +  
Leu221Cys + Ser222Glu + Thr224Asn  
Ala204Asn + Gly206Pro + Ser207Asp + Trp208His +  
Ile209His + Pro214Gln + Leu221Gln  
Ala204Asn + Gly206Asn + Trp208Ile + Ile209Asn +  
Ser216Glu + Ala219Pro + Gly223Asn  
Ala204Ser + Gly206Ser + Ile209Ala + Thr212Ser +  
Pro214Gln + Tyr218Asp + Leu221His  
Gly206Asn + Ile209Pro + Tyr213Cys + Thr215Gln +

Ser216Glu + Thr217Pro + Ala219His  
Ala204Gln + Trp208His + Tyr210Gly + Thr212Gln +  
Tyr218Met + Ala219Asn + Ser220Glu  
Ala204Pro + Ser207Asp + Tyr213Val + Pro214Gly +  
Thr215Gly + Tyr218Ala + Leu221Gln  
Pro205Asn + Gly206Pro + Trp208Leu + Tyr210Pro +  
Pro214Asn + Thr217Gln + Tyr218Glu  
Pro205Asn + Gly206Gln + Tyr213Val + Ala219Gln +  
Ser220Asp + Gly223Ser + Thr224Asn  
Gly206Pro + Ile209Cys + Tyr210Ser + Thr212Asn +  
Tyr213Cys + Pro214Gln + Tyr218Cys  
Ala204His + Trp208Asn + Thr212Pro + Pro214Gln +  
Thr215Asn + Thr217Gly + Thr224Asp  
Pro205Ser + Gly206Ser + Tyr210Ser + Thr215Pro +  
Ala219Asn + Leu221Met + Ser222Asp  
Ala204Ser + Gly206Gln + Trp208Pro + Thr212Asn +  
Thr215Glu + Tyr218Ile + Gly223Asn  
Ala204Ser + Pro205Asn + Trp208Tyr + Thr215Gly +  
Tyr218Pro + Ser220Glu + Thr224Gly  
Ala204Asn + Tyr210His + Pro214Asn + Tyr218Pro +  
Ala219Gln + Ser220Glu + Leu221Ile  
Ala204Gly + Gly206Asn + Ser207Asp + Thr212Asn +  
Tyr213Leu + Thr215Pro + Gly223Pro  
Ala204Asn + Tyr210Met + Thr212Gln + Pro214Gly +  
Ala219Gly + Ser222Asp + Gly223Gln  
Tyr210Pro + Thr212Gly + Tyr213Leu + Thr215Gly +  
Thr217Gly + Ala219Glu + Ser220Asp  
Gly206Ser + Trp208Asn + Ile209Pro + Ala219Asp +  
Ser220Asp + Gly223Ser + Thr224Gly  
Pro205Ser + Tyr210Cys + Tyr213Thr + Pro214Asp +  
Thr215Asp + Tyr218Val + Leu221Ser  
Trp208His + Ile209Val + Thr212Pro + Tyr213Leu +  
Thr217Ser + Ser222Glu + Gly223Glu  
Pro205Gly + Gly206Gln + Tyr210Asn + Tyr213Gly +  
Thr217Gly + Ser222Glu + Gly223Glu  
Ala204Thr + Thr212Gln + Tyr213Gln + Thr217Ser +  
Ser222Asp + Gly223Asp + Thr224Pro  
Pro205Gly + Ile209Cys + Tyr213Val + Pro214Gln +  
Thr215Pro + Ser222Glu + Gly223Asp  
Trp208Ser + Ile209Ser + Ser216Glu + Thr217Glu +  
Tyr218Gly + Ala219His + Gly223Pro  
Gly206Pro + Tyr210Gln + Thr212Pro + Tyr213Leu +  
Ser216Glu + Thr217Asp + Leu221Pro  
Trp208Met + Ile209Gln + Tyr210Met + Tyr218Gln +  
Ser220Glu + Leu221Glu + Thr224Ser  
Ile209Gln + Thr212Ser + Tyr213Val + Thr215Asp +  
Ser216Glu + Tyr218Met + Ala219Pro  
Tyr213Pro + Pro214Gln + Thr215Asp + Ser216Glu +  
Thr217Asp + Tyr218Met + Leu221Ala  
Ala204His + Pro205Gly + Ser207Glu + Trp208His +  
Tyr210His + Tyr213Cys + Ser222Asp  
Gly206Pro + Ser207Asp + Trp208Ser + Pro214Ser +  
Leu221Asn + Ser222Asp + Gly223Pro

Ser207Glu + Trp208Tyr + Ile209Gly + Tyr213Thr +  
Pro214Ser + Tyr218Met + Ser222Glu  
Pro205Ser + Ser207Asp + Trp208Ser + Thr212Gly +  
Tyr213Asn + Tyr218Gln + Ser222Asp  
Ala204Thr + Gly206Asn + Trp208Thr + Thr212Gln +  
Pro214Glu + Thr215Asp + Ser216Asp  
Ala204Thr + Gly206Asn + Ser207Glu + Tyr210Ile +  
Leu221Thr + Ser222Glu + Gly223Glu  
Ala204His + Ser207Glu + Thr215Gln + Thr217Gln +  
Tyr218Asn + Ser222Glu + Gly223Glu  
Ala204Gln + Trp208Asp + Tyr210Thr + Thr212Ser +  
Thr217Asn + Ser222Asp + Gly223Asn  
Ile209Thr + Ser216Asp + Thr217Asp + Tyr218Asp +  
Leu221Ser + Gly223Ser + Thr224Gly  
Ala204Asn + Gly206Gln + Ser207Glu + Trp208His +  
Thr212Ser + Leu221Asp + Ser222Asp  
Gly206Ser + Ser207Asp + Tyr210Val + Thr212Gln +  
Tyr218Ile + Leu221Asp + Ser222Asp  
Ala204Ser + Gly206Pro + Pro214Gly + Thr215Asp +  
Thr217Asp + Tyr218Pro + Gly223Pro  
Gly206Gln + Ile209His + Pro214Asp + Thr215Pro +  
Ser216Asp + Leu221Gly + Thr224Ser  
Gly206Asn + Ser207Glu + Trp208Asp + Ile209Gly +  
Tyr213Gly + Ser220Glu + Ser222Glu  
Pro205Ser + Ser207Asp + Ile209Asn + Tyr213Met +  
Thr217Gln + Ala219His + Gly223Glu  
Pro205Gly + Gly206Gln + Ser207Glu + Ala219Pro +  
Ser220Asp + Leu221Glu + Thr224Ser  
Trp208Met + Tyr210Thr + Thr212Asn + Ala219Gln +  
Leu221Asp + Gly223Asp + Thr224Gln  
Pro205Ser + Ser207Glu + Tyr213His + Thr217Pro +  
Ala219Ser + Ser220Glu + Ser222Glu  
Ser207Asp + Tyr210Met + Pro214Ser + Tyr218Asn +  
Ser220Glu + Leu221Cys + Ser222Glu  
Ala204Pro + Ser207Glu + Pro214Gln + Ser220Asp +  
Leu221His + Ser222Asp + Thr224Ser  
Ser207Glu + Ile209Met + Tyr210Gly + Tyr213Leu +  
Thr215Gln + Ser220Glu + Ser222Asp  
Gly206Pro + Ser207Asp + Ile209Ser + Tyr210His +  
Thr217Gly + Ser222Glu + Thr224Asp  
Pro205Asn + Tyr210Cys + Tyr213Gly + Thr217Asn +  
Tyr218Met + Ser220Glu + Ser222Asp  
Ile209Gly + Thr212Pro + Thr215Asn + Thr217Gly +  
Ala219His + Ser220Glu + Ser222Glu  
Ala204Gln + Gly206Gln + Thr212Pro + Ser220Glu +  
Leu221Val + Ser222Asp + Thr224Gln  
Ala204Gln + Pro205Ser + Gly206Ser + Trp208Gln +  
Thr215Pro + Ser220Glu + Ser222Glu  
Ser207Glu + Trp208Leu + Tyr210Ile + Thr212Gly +  
Tyr218Cys + Leu221Asp + Gly223Asn  
Pro205Gly + Gly206Pro + Ser207Asp + Ile209Met +  
Leu221Asp + Ser222Glu + Thr224Asp  
Pro205Gly + Thr215Asn + Thr217Pro + Tyr218Val +

Leu221Thr + Ser222Glu + Thr224Asp  
Trp208Phe + Tyr213Met + Thr215Asn + Tyr218Asp +  
Ala219Pro + Ser220Glu + Thr224Gly  
Ala204Pro + Pro205Gly + Trp208Leu + Thr217Gln +  
Leu221Asp + Ser222Glu + Thr224Asp  
Ile209Ala + Thr212Gln + Thr217Gln + Tyr218Thr +  
Ser220Glu + Leu221Asp + Gly223Glu  
Ala204Asn + Ile209Cys + Tyr213Cys + Tyr218Ala +  
Ala219Glu + Leu221Asp + Ser222Glu  
Gly206Ser + Ser207Glu + Trp208His + Tyr210Asp +  
Tyr218Val + Ala219Thr + Ser222Asp  
Pro205Gly + Ser207Asp + Trp208Thr + Thr212Ser +  
Thr217Gly + Ser220Glu + Gly223Ser  
Ser207Glu + Trp208Asn + Tyr210Pro + Pro214Asn +  
Tyr218Ser + Ser220Asp + Gly223Ser  
Ala204Gln + Ser207Asp + Trp208Gly + Ile209Ala +  
Thr212Asn + Ser220Glu + Gly223Pro  
Pro205Gln + Ser207Asp + Ile209Gln + Pro214Asn +  
Thr215Gln + Ser220Asp + Thr224Gly  
Pro205Asn + Ser207Glu + Ile209Val + Thr212Gly +  
Tyr213Pro + Ala219Asn + Thr224Glu  
Ala204Gly + Ser207Glu + Ile209Ala + Thr212Pro +  
Tyr213Cys + Ala219Glu + Ser220Asp  
Pro205Ser + Trp208Glu + Thr212Asn + Tyr218Glu +  
Ala219His + Leu221Glu + Ser222Glu  
Pro205Ser + Ile209Pro + Tyr210Asp + Thr212Ser +  
Tyr213Ile + Ala219Pro + Ser222Glu  
Gly206Ser + Trp208Thr + Tyr210Ala + Pro214Asp +  
Tyr218Met + Ala219Glu + Ser220Asp  
Ile209Leu + Pro214Glu + Thr215Pro + Ala219Glu +  
Ser220Glu + Gly223Pro + Thr224Gly  
Trp208Tyr + Thr212Asn + Thr217Glu + Ala219Gly +  
Ser220Asp + Leu221Glu + Ser222Asp  
Ala204Thr + Tyr210Pro + Tyr213Asn + Pro214Glu +  
Tyr218Asp + Ser220Asp + Thr224Gly  
Gly206Pro + Thr212Pro + Pro214Gly + Thr215Pro +  
Ser220Asp + Leu221Asp + Thr224Glu  
Thr212Asn + Pro214Gln + Tyr218Met + Ala219Asp +  
Ser222Glu + Gly223Asp + Thr224Glu  
Trp208Gly + Tyr210Pro + Thr212Gly + Ser220Glu +  
Leu221Met + Gly223Asp + Thr224Glu  
Gly206Gln + Tyr213Asn + Pro214Asn + Thr217Asp +  
Ala219His + Ser220Glu + Leu221Asp  
Pro205Gln + Ile209Pro + Tyr210Asn + Tyr218Thr +  
Ala219Glu + Ser220Asp + Gly223Asp  
Ala204Thr + Ser207Asp + Tyr210Asp + Tyr213Met +  
Ala219Asn + Ser220Glu + Thr224Asp  
Pro205Ser + Trp208Pro + Ile209Pro + Tyr213Ile +  
Ala219Asp + Ser222Asp + Gly223Asp  
Ala204Asn + Thr212Pro + Thr217Glu + Tyr218Asp +  
Ser220Glu + Ser222Asp + Thr224Gln  
Gly206Pro + Ser207Asp + Trp208Val + Tyr210Thr +  
Tyr213Ala + Ser220Asp + Thr224Asp



Pro205Gly + Ser207Glu + Tyr210Ile + Pro214Ser +  
Thr217Gly + Ser220Asp + Thr224Glu  
Ala204Thr + Tyr210Cys + Thr212Pro + Tyr218Ile +  
Ser220Glu + Leu221Ser + Gly223Glu  
Ala204Gln + Trp208Pro + Pro214Asn + Ala219Thr +  
Ser220Glu + Gly223Asp + Thr224Gln  
Pro205Gln + Tyr213Gly + Thr215Gly + Tyr218Ala +  
Ser220Asp + Gly223Glu + Thr224Pro  
Pro205Gly + Gly206Ser + Thr217Pro + Ala219His +  
Ser220Asp + Leu221Cys + Gly223Asp  
Pro205Ser + Tyr210Thr + Thr212Asn + Tyr213Cys +  
Pro214Ser + Ala219Asp + Ser222Asp  
Trp208Asn + Thr217Glu + Tyr218Cys + Ala219Glu +  
Ser220Asp + Gly223Glu + Thr224Ser  
Pro205Ser + Gly206Pro + Trp208Pro + Ile209Ala +  
Thr215Asp + Tyr218Asp + Ser220Asp  
Trp208Thr + Thr212Gly + Tyr213Ile + Thr215Asp +  
Leu221Asp + Ser222Glu + Gly223Glu  
Ala204Gly + Pro205Gly + Tyr210Glu + Thr217Glu +  
Tyr218Cys + Ala219Gly + Leu221Val  
Ser207Asp + Thr215Pro + Thr217Asp + Tyr218Ile +  
Leu221Ala + Ser222Glu + Gly223Asp  
Gly206Pro + Trp208Glu + Tyr210Cys + Pro214Gln +  
Thr217Asp + Ser220Asp + Gly223Gln  
Trp208Thr + Tyr213Pro + Pro214Ser + Ser216Asp +  
Tyr218Met + Ala219Asp + Thr224Pro  
Ala204Asn + Trp208Ala + Tyr213Asn + Ser216Asp +  
Ala219Glu + Leu221Cys + Thr224Gly  
Gly206Ser + Tyr210Ser + Pro214Gly + Ser216Asp +  
Tyr218Cys + Ala219Glu + Leu221Pro  
Ala204Thr + Tyr210Gln + Thr212Gln + Tyr213Ala +  
Pro214Gln + Thr217Asp + Ser220Glu  
Pro205Gln + Gly206Ser + Ser207Glu + Tyr210Ser +  
Ser216Asp + Thr217Glu + Tyr218Glu  
Tyr210Met + Tyr213Met + Ser216Asp + Ala219Glu +  
Ser220Glu + Leu221Cys + Ser222Glu  
Ala204Gln + Ile209His + Ser216Glu + Thr217Ser +  
Ala219Glu + Ser220Asp + Ser222Glu  
Ile209Ser + Tyr210Leu + Tyr213Leu + Pro214Asp +  
Thr217Asp + Ser220Glu + Leu221Glu  
Ala204His + Pro205Gln + Pro214Asn + Tyr218Gln +  
Ala219Asp + Leu221Glu + Thr224Asp  
Ala204Gly + Ser207Glu + Pro214Glu + Tyr218Pro +  
Ser220Glu + Ser222Asp + Thr224Asn  
Ala204Pro + Pro205Gly + Trp208Glu + Ile209Ser +  
Tyr210Asp + Ala219Thr + Thr224Glu  
Ala204Gly + Ser207Glu + Trp208Glu + Tyr210Asp +  
Ser216Glu + Thr217Gln + Thr224Gly  
Trp208Ala + Tyr210Cys + Thr212Ser + Thr217Glu +  
Ala219Gln + Ser220Asp + Ser222Glu  
Gly206Asn + Ser207Glu + Trp208Glu + Thr212Ser +  
Ser216Glu + Leu221Met + Gly223Glu  
Ala204Thr + Ser207Asp + Ile209Cys + Thr215Asp +

Tyr218Ile + Ser220Asp + Leu221Asp  
Ser207Asp + Thr212Pro + Pro214Gln + Ser216Glu +  
Tyr216Val + Ser220Asp + Ser222Asp  
Ile209Pro + Thr212Gly + Tyr213Ser + Pro214Glu +  
Thr215Glu + Ala219Gln + Ser220Glu  
Pro205Asn + Ser207Glu + Ile209Met + Pro214Ser +  
Ser216Asp + Ala219Glu + Leu221Asp  
Ala204Thr + Ser207Glu + Tyr210Leu + Ser216Glu +  
Ala219Asp + Leu221Asp + Gly223Asn  
Gly206Asn + Ser207Glu + Pro214Gly + Thr215Glu +  
Thr217Glu + Tyr218Thr + Ala219Glu  
Gly206Gln + Ser207Glu + Tyr210Ala + Thr215Asn +  
Thr217Asp + Ser220Asp + Leu221Ala  
Ser207Glu + Trp208Leu + Tyr213Val + Pro214Asp +  
Ala219Glu + Ser222Glu + Thr224Pro  
Ala204Thr + Ser207Asp + Trp208Leu + Tyr210Asp +  
Thr212Pro + Thr215Asp + Leu221Glu  
Pro205Gln + Ser207Asp + Ile209Ala + Thr217Asn +  
Tyr218Glu + Ala219Asp + Gly223Asp  
Ser207Glu + Ile209Thr + Tyr213Gly + Ser216Asp +  
Ala219Asp + Ser222Glu + Gly223Pro  
Gly206Ser + Ser207Asp + Ile209Ala + Thr212Gln +  
Thr217Glu + Ala219Glu + Leu221Asn  
Pro205Asn + Tyr210Glu + Thr217Asp + Tyr218Ala +  
Leu221Ile + Ser222Glu + Thr224Pro  
Ser216Asp + Thr217Asp + Tyr218Cys + Ala219His +  
Leu221Glu + Ser222Glu + Gly223Asn  
Ala204Gln + Trp208Asp + Pro214Gln + Ser216Glu +  
Thr217Gln + Ser220Asp + Gly223Asp  
Pro205Gln + Tyr210Leu + Tyr213Thr + Ser216Glu +  
Tyr218Glu + Ser220Glu + Gly223Glu  
Pro214Gly + Thr217Asp + Tyr218Gly + Ala219Glu +  
Leu221Asn + Ser222Glu + Thr224Glu  
Gly206Pro + Ser207Asp + Trp208Asp + Thr212Gln +  
Thr217Asp + Gly223Ser + Thr224Glu  
Ala204Pro + Pro205Ser + Gly206Pro + Ile209Val +  
Tyr213Pro + Thr215Asp + Ala219Glu  
Ala204Pro + Gly206Pro + Tyr210Val + Pro214Glu +  
Ser220Glu + Ser222Glu + Thr224Pro  
Ala204Gly + Gly206Pro + Thr212Gln + Pro214Glu +  
Ser220Glu + Leu221Val + Ser222Asp  
Ala204Asn + Gly206Gln + Ile209Ser + Tyr210Ala +  
Pro214Glu + Ser220Glu + Ser222Asp  
Pro205Gln + Trp208Gln + Pro214Glu + Thr217Pro +  
Leu221Met + Ser222Asp + Gly223Glu  
Ala204Thr + Pro205Asn + Pro214Asp + Thr215Pro +  
Leu221Cys + Ser222Asp + Gly223Asp  
Ile209Pro + Tyr210Pro + Thr212Pro + Thr217Asp +  
Leu221Asn + Ser222Asp + Gly223Asp  
Gly206Gln + Trp208Thr + Ile209Ser + Tyr213Met +  
Thr215Glu + Ser222Asp + Gly223Glu  
Pro205Asn + Trp208Ser + Thr212Gly + Tyr213Asn +  
Thr215Asp + Ser222Asp + Gly223Glu

Tyr210Gly + Thr212Pro + Pro214Gly + Ser216Asp +  
 Ala219His + Leu221Glu + Ser222Asp  
 Pro205Gly + Gly206Pro + Ile209His + Thr215Asp +  
 Ala219Gly + Leu221Glu + Ser222Glu  
 5 Pro205Gln + Tyr210Gly + Thr212Asn + Ser216Asp +  
 Ala219Pro + Ser220Glu + Leu221Asp  
 Gly206Ser + Trp208Glu + Tyr210Gln + Thr215Gly +  
 Ser216Glu + Thr217Glu + Leu221Pro  
 Pro205Gln + Gly206Gln + Ser216Glu + Thr217Asp +  
 10 Leu221Ala + Ser222Glu + Thr224Pro  
 Gly206Ser + Ser207Glu + Trp208Asp + Ile209Leu +  
 Thr215Asp + Ser216Glu + Tyr218Gln  
 Tyr210His + Thr212Gln + Thr215Asp + Ser216Glu +  
 Leu221Glu + Gly223Ser + Thr224Pro  
 15 Ala204Ser + Pro205Ser + Gly206Asn + Thr215Asp +  
 Ser216Glu + Tyr218Val + Gly223Asp  
 Ala204Gln + Gly206Asn + Ser207Glu + Trp208Pro +  
 Pro214Asp + Thr215Asp + Ser222Asp  
 Ser207Asp + Tyr210Gln + Thr212Gln + Thr215Asp +  
 20 Ser216Asp + Leu221Gly + Ser222Asp  
 Ile209Met + Tyr210Leu + Thr217Glu + Tyr218Thr +  
 Ser220Glu + Gly223Glu + Thr224Asn  
 Pro205Ser + Ser207Glu + Trp208His + Thr215Pro +  
 Thr217Asp + Leu221Asn + Ser222Glu  
 25 Ala204Thr + Ser207Glu + Thr212Gly + Tyr213Leu +  
 Pro214Asn + Ser216Glu + Ser222Glu  
 Ala204Pro + Ser207Asp + Tyr210Leu + Thr212Gln +  
 Tyr213Thr + Thr215Glu + Ser222Glu

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TABLE 29

## Multi-loop Double Mutation Variants

	Leu104Gly + Tyr210Pro
	Asn 66Gln + Thr217Glu
	Gly 67Ser + Gly 69Ser
35	Val103Gln + Gly223Asp
	Trp112Cys + Thr217Gly
	Ala164Glu + Trp208Gln
	Leu134Ile + Trp208Gln
	Ala219Gly + Ser220Glu
40	Asn 66Asp + Leu104Gly
	Ala168Thr + Ser216Glu
	Leu104Ala + Ser109Glu
	Thr111Gln + Val115Ala
	Ser109Asp + Tyr210Pro
45	Gly 69Ser + Ser191Asp
	Val103Asp + Gly206Gln
	Gly 67Glu + Pro214Asn
	Trp208Ile + Thr215Asp
	Ser107Glu + Thr113Gly
50	Gly108Ser + Leu221Glu
	Ser 70Glu + Pro169Asn

Gly165Gln + Leu221Asp  
Gly108Glu + Ala164Gly  
Leu104His + Thr167Pro  
Ala164His + Ser220Glu  
Thr195Gln + Ser222Asp  
Trp208His + Ala219Pro  
Gly 69Glu + Asn106Gln  
Trp112Cys + Thr113Pro  
Gly 67Gln + Trp208Asp  
Ala114Glu + Leu134Ile  
Ser109Glu + Gly136Ser  
Gly110Ser + Ser207Glu  
Val103Ala + Thr212Ser  
Ala114Glu + Thr167Pro  
Thr195Glu + Ala204Thr  
Gly 67Ser + Thr217Glu  
Trp208Tyr + Ser220Glu  
Ala168His + Thr217Asp  
Ala168Glu + Tyr213Asn  
Gly110Pro + Ser220Asp  
Tyr171Asn + Leu221Met  
Thr 73Asp + Leu134Ser  
Thr137Asn + Thr217Pro  
Ser 70Asp + Ala168Thr  
Thr167Glu + Tyr210Pro  
Thr195Glu + Tyr210Gly  
Tyr210Gln + Thr217Glu  
Gly 67Glu + Val115Cys  
Ser 70Glu + Ala219Thr  
Gly136Pro + Leu221Ala  
Gly 67Ser + Thr195Ser  
Phe193His + Ser222Asp  
Asn 68Glu + Tyr210Ser  
Gly165Asp + Pro169Ser  
Trp208Pro + Thr215Gly  
Thr 73Gly + Leu104Gly  
Thr113Asn + Ser216Glu  
Gly 72Gln + Gly139Asp  
Tyr210Ile + Leu221Gly  
Thr 73Pro + Tyr171Asp  
Leu104Gln + Thr137Gly  
Gly 69Pro + Tyr210Ala  
Thr217Gly + Gly223Glu  
Thr195Asp + Tyr213Ser  
Gly 72Asn + Ala219Asn  
Val138Pro + Tyr210Ala  
Tyr218Met + Gly223Asn  
Ser220Glu + Leu221Asn  
Trp112Ala + Gly139Asn  
Gly135Glu + Leu221Gly  
Thr113Asn + Thr217Ser  
Thr195Asn + Thr217Asp  
Tyr210Val + Ser220Glu

Vall138Gly + Ala219Asp  
Asn166Glu + Leu221His  
Gly165Gln + Leu221His  
Thr113Gln + Ser192Asp  
Asn 66Asp + Thr 73Ser  
Thr167Gln + Thr217Gly  
Vall115Pro + Gly135Ser  
Ala219Gln + Gly223Asp  
Trp208His + Thr217Asn  
Trp208Leu + Tyr210Val  
Gly108Asn + Ser191Glu  
Ser107Glu + Tyr210Ala  
Asn 68Gln + Ser220Glu  
Trp112Cys + Leu221Asn  
Gly108Gln + Ser109Asp  
Vall103Met + Ala168His  
Ala164Pro + Ser192Glu  
Pro214Ser + Ser220Glu  
Thr215Asn + Thr217Gln  
Ser220Glu + Leu221Pro  
Gly136Ser + Trp208Phe  
Thr195Asp + Tyr210Gln  
Leu104Gln + Ala219Asp  
Gly 69Pro + Leu221Pro  
Gly 69Asn + Ala204Gly  
Gly135Glu + Ala219Asn  
Gly136Asn + Ser220Glu  
Gly136Ser + Trp208Val  
Gly139Asp + Asn140Gln  
Trp208Cys + Tyr210Asn  
Gly165Ser + Ser220Asp  
Gly110Ser + Trp208Thr  
Ala114Asp + Gly162Asn  
Ser192Asp + Leu221Cys  
Vall103Ser + Gly135Glu  
Asn140Glu + Trp208Ala  
Thr113Asp + Leu221Ser  
Ser109Asp + Gly110Ser  
Ser109Glu + Thr113Gln  
Thr 73Gly + Gly136Asp  
Ala164His + Leu221Asn  
Gly 67Pro + Vall103His  
Ala114Asn + Thr212Gln  
Asn140Asp + Gly223Pro  
Gly139Gln + Pro214Asn  
Asp105Glu + Thr195Gly  
Gly135Pro + Ser207Glu  
Leu104Glu + Ala114Pro  
Asn 66Glu + Gly 69Pro  
Vall115Cys + Ser207Glu  
Asn 68Glu + Leu221Ala  
Asn 66Ser + Gly 69Asp  
Ser109Glu + Phe193Pro

Trp208Glu + Thr212Pro  
Gly136Gln + Trp208Cys  
Ser 70Asp + Leu221His  
Ser 70Glu + Phe193Ser  
Thr217Asn + Ser222Glu  
Tyr213Thr + Tyr218Gly  
Trp208Ser + Leu221Cys  
Asn163Asp + Leu221Ser  
Asn 66Gln + Gly223Pro  
Gly139Asn + Ser220Asp  
Thr 73Ser + Pro214Asp  
Ser 70Asp + Leu221Ile  
Gly165Gln + Ser220Asp  
Asn166Asp + Thr217Asn  
Asp105Glu + Thr111Pro  
Asn140Asp + Trp208Phe  
Asn166Glu + Pro214Asn  
Trp208Ala + Thr217Ser  
Gly108Ser + Gly110Glu  
Val138Asn + Asn170Asp  
Gly110Glu + Thr224Gln  
Trp208Met + Ser220Asp  
Asn 68Glu + Val115Ser  
Asn170Glu + Tyr210Gly  
Gly110Asn + Ser192Glu  
Trp208Gln + Ser220Glu  
Asn140Gln + Pro214Gln  
Thr217Ser + Ser220Glu  
Ser141Glu + Ala168Thr  
Gly162Glu + Tyr210His  
Leu104Gly + Thr224Asp  
Trp112Pro + Ser191Asp  
Trp208Thr + Tyr210Glu  
Gly108Gln + Thr111Asp  
Leu134Val + Thr217Pro  
Asn170Asp + Tyr210His  
Val115Ser + Ser220Glu  
Gly 69Pro + Ser 70Asp  
Thr 73Gly + Tyr210Glu  
Gly 67Asp + Gly108Gln  
Gly 69Ser + Gly110Asp  
Tyr210Thr + Ser220Glu  
Trp208Phe + Leu221Met  
Gly136Gln + Leu221Asp  
Thr113Ser + Trp208Val  
Tyr171Leu + Tyr210Thr  
Leu134Asn + Thr167Gln  
Asn140Ser + Tyr210Asp  
Asn 66Gln + Gly 67Glu  
Leu134Cys + Pro214Asn  
Gly136Ser + Leu221Ala  
Thr111Pro + Trp208Val  
Thr113Asp + Tyr171Gly

Trp208Ile + Ser220Glu  
Pro214Ser + Thr224Glu  
Gly165Pro + Ser220Glu  
Thr137Gln + Tyr171Glu  
Trp208Glu + Pro214Gly  
Ser216Asp + Leu221Met  
Gly136Ser + Thr217Gln  
Ser141Glu + Tyr210Leu  
Trp208Thr + Gly223Ser  
Asn 66Ser + Asn170Gln  
Asn 66Gln + Asn170Glu  
Pro169Glu + Tyr210Thr  
Tyr171Leu + Leu221Asp  
Gly139Glu + Leu221Pro  
Thr 73Pro + Leu134Gly  
Tyr210Glu + Leu221Ala  
Gly139Ser + Tyr210Gly  
Asn163Asp + Ala164Ser  
Asn163Gln + Thr217Asp  
Asn140Asp + Tyr218Ser  
Gly108Ser + Ala164Gln  
Val138Ser + Trp208Leu  
Thr217Ser + Thr224Asp  
Ser109Asp + Tyr218Ala  
Asn 66Glu + Phe193Val  
Asn140Asp + Ala204Gly  
Val138Ser + Ser220Asp  
Gly108Asn + Tyr210Thr  
Gly139Asn + Tyr171Pro  
Thr113Pro + Trp208Thr  
Ser141Glu + Leu221Gly  
Asn106Glu + Thr167Gly  
Trp112Ser + Ala219Ser  
Asp105Glu + Asn166Gln  
Ala114Pro + Tyr171Asn  
Thr217Gln + Ser220Asp  
Thr113Gly + Tyr210Asp  
Tyr171Cys + Tyr210Glu  
Asn166Asp + Leu221Val  
Ala114His + Ser191Asp  
Gly 67Glu + Thr217Asn  
Asn 66Glu + Thr215Gln  
Thr111Asn + Ser220Glu  
Trp208Gln + Leu221Ser  
Gly 69Gln + Ala114Thr  
Asn163Asp + Leu221Ala  
Pro214Gly + Leu221Ile  
Thr217Gly + Ser220Asp  
Ala219Thr + Ser220Asp  
Tyr210Cys + Ser220Glu  
Val138Asp + Gly223Pro  
Thr195Asn + Leu221Gly  
Leu104Thr + Thr215Ser

Gly 69Gln + Leu134Thr  
Gly165Pro + Leu221Gly  
Thr167Ser + Thr217Glu  
Thr212Pro + Thr217Glu  
Asn166Ser + Tyr218Asp  
Gly108Glu + Tyr171Gln  
Gly 67Asn + Tyr210Ile  
Val115Cys + Pro214Gly  
Thr137Asp + Tyr210Val  
Leu104Met + Trp208Gly  
Leu134Ala + Ser192Asp  
Trp112Gln + Ala219Gln  
Gly108Glu + Val115His  
Thr113Asn + Gly139Pro  
Trp208Gln + Gly223Asn  
Ser207Glu + Trp208Gln  
Asn 66Ser + Tyr210Asn  
Ser207Glu + Tyr210Pro  
Val103Ala + Thr215Glu  
Val103Asn + Tyr210Ala  
Asn 66Ser + Trp208Phe  
Thr215Asn + Ala219Thr  
Gly136Asn + Gly139Asn  
Tyr171Thr + Ser216Glu  
Leu104Glu + Tyr210Asn  
Ser109Glu + Thr217Asn  
Leu134Ile + Asn166Ser  
Tyr171Gly + Thr217Gln  
Val115Cys + Thr224Asp  
Pro169Ser + Thr215Asp  
Gly 72Gln + Gly135Asn  
Tyr210Thr + Ser220Asp  
Asn 68Glu + Thr113Ser  
Asn106Gln + Thr113Asp  
Ser109Glu + Trp208Asn  
Leu134Gln + Thr137Glu  
Ala114Thr + Asn170Glu  
Ala219Pro + Ser220Glu  
Tyr171Val + Trp208Pro  
Gly139Ser + Tyr213Ser  
Ser107Asp + Thr113Asn  
Thr167Gly + Leu221Met  
Tyr171Gly + Ser207Glu  
Gly 67Ser + Thr217Ser  
Val115Gly + Thr217Ser  
Asn 66Ser + Trp208Ile  
Ser191Glu + Trp208Cys  
Thr137Asp + Trp208Ser  
Leu221Gly + Ser222Glu  
Ser216Glu + Leu221Thr  
Gly162Pro + Ser207Glu  
Thr217Asp + Ala219Thr  
Leu104Cys + Ser207Glu



Gly 69Gln + Ser220Glu  
Gly 69Gln + Val138Asp  
Pro169Gly + Tyr210Leu  
Thr 73Asp + Gly110Ser  
Thr215Gly + Thr217Ser  
Gly 69Ser + Gly 72Asn  
Ser191Glu + Thr217Asn  
Gly135Ser + Tyr171Ser  
Thr111Asn + Phe193Leu  
Trp112Leu + Ser207Asp  
Leu104Glu + Trp208Ala  
Leu221Ile + Thr224Asp  
Gly136Glu + Gly165Asn  
Tyr210Glu + Thr217Pro  
Trp112Phe + Pro214Asp  
Gly110Gln + Trp112Met  
Asn 68Ser + Trp208Asp  
Gly 69Ser + Leu104Cys  
Thr137Glu + Thr215Pro  
Asn166Gln + Thr217Glu  
Tyr210Thr + Thr212Ser  
Leu134His + Trp208Asp  
Ser141Glu + Tyr210Val  
Ser191Glu + Tyr210Leu  
Gly139Pro + Ser207Asp  
Gly162Ser + Tyr171Met  
Thr 73Gln + Val103Ala  
Tyr171Val + Phe193Asp  
Leu221Thr + Ser222Glu  
Gly 69Asp + Ala114Gln  
Gly108Glu + Trp208Met  
Gly 72Ser + Tyr210Gln  
Thr137Asn + Trp208Asp  
Asn140Asp + Phe193Thr  
Ser192Glu + Trp208Ala  
Asn170Glu + Pro205Ser  
Tyr210Ala + Tyr213Ser  
Tyr171Gln + Tyr218Ile  
Asn 66Asp + Leu221Cys  
Asn163Gln + Gly223Asn  
Val103Thr + Leu134Ser  
Thr167Ser + Asn170Asp  
Ser 70Glu + Gly162Ser  
Tyr171Gln + Ser192Glu  
Tyr210Val + Gly223Ser  
Thr137Glu + Leu221Pro  
Gly 69Asn + Thr217Asp  
Pro205Ser + Tyr218Glu  
Tyr210Met + Leu221Asn  
Thr215Asp + Thr217Pro  
Leu134Val + Gly136Glu  
Thr 73Pro + Leu221Glu  
Tyr213Val + Ser220Glu

Thr 73Ser + Ser107Glu  
 Trp208Ser + Leu221Pro  
 Thr113Asp + Leu221His  
 Ala114Thr + Thr217Asp  
 Ala168Ser + Tyr218Glu  
 Trp208Asp + Leu221Asn  
 Thr137Asp + Trp208Ile  
 Gly135Gln + Ser191Glu  
 Val103Glu + Trp208Gly  
 Asn140Asp + Leu221His  
 Ser192Asp + Tyr210Cys  
 Gly108Asn + Tyr210His  
 Asp105Glu + Trp208Asn  
 Val103Pro + Tyr210Pro  
 Thr 73Gln + Trp112Tyr  
 Leu134Cys + Asn170Ser  
 Asn166Glu + Tyr210Cys  
 Asn106Ser + Leu221Met  
 Ser109Glu + Leu221Cys  
 Ser107Asp + Leu221Ala  
 Ala114Ser + Leu221Asn  
 Gly162Asn + Leu221Val

TABLE 33

## Multi-loop Triple Mutation Variants

Leu104Gly + Tyr210Pro + Thr217Glu  
 Asn 66Gln + Gly 67Ser + Gly 69Ser  
 Val103Gln + Trp112Cys + Gly223Asp  
 Ala164Glu + Trp208Gln + Thr217Gly  
 Leu134Ile + Trp208Gln + Ser220Glu  
 Asn 66Asp + Leu104Gly + Ala219Gly  
 Leu104Ala + Thr111Gln + Val115Ala  
 Gly 69Ser + Ser109Asp + Tyr210Pro  
 Gly139Asn + Thr167Asn + Thr217Ser  
 Gly108Glu + Ala164Gly + Gly165Gln  
 Leu104His + Thr167Pro + Ser220Asp  
 Gly 69Glu + Asn106Gln + Trp208His  
 Trp112Cys + Thr113Pro + Trp208Asp  
 Gly110Ser + Ser207Glu + Thr212Ser  
 Ala168His + Trp208Tyr + Ser220Glu  
 Tyr171Asn + Thr217Asp + Leu221Met  
 Ser 70Asp + Thr137Asn + Thr217Pro  
 Thr167Glu + Ala168Thr + Tyr210Pro  
 Ser 70Glu + Gly136Pro + Leu221Ala  
 Gly 67Ser + Thr195Ser + Ser222Asp  
 Pro169Ser + Trp208Pro + Thr215Gly  
 Thr 73Gly + Leu104Gly + Tyr171Glu  
 Gly 72Gln + Tyr210Ile + Leu221Gly  
 Leu104Gln + Thr137Gly + Tyr210Ala  
 Tyr213Ser + Thr217Gly + Gly223Glu  
 Gly 72Asn + Thr195Asp + Ala219Asn

Thr137Asp + Val138Pro + Tyr210Ala  
Ser109Glu + Val115Met + Trp208Cys  
Gly139Asn + Ser220Glu + Leu221Asn  
Trp112Ala + Gly135Glu + Leu221Gly  
Thr113Asn + Thr195Asn + Thr217Ser  
Ala164His + Ser220Asp + Leu221His  
Asn 66Asp + Thr 73Ser + Thr113Gln  
Gly135Ser + Thr167Gln + Thr217Gly  
Tyr171Asp + Trp208His + Thr217Asn  
Gly108Asn + Trp208Leu + Tyr210Val  
Trp112Cys + Ser220Glu + Leu221Asn  
Val103Met + Ala168His + Ser191Asp  
Pro214Ser + Thr217Gln + Ser220Glu  
Thr215Asn + Ser220Glu + Leu221Pro  
Gly136Ser + Trp208Phe + Tyr210Gln  
Gly 69Pro + Ala219Asp + Leu221Pro  
Gly 69Asn + Ala204Gly + Ser220Asp  
Gly136Ser + Gly139Asp + Trp208Val  
Asn140Gln + Trp208Cys + Tyr210Asn  
Gly110Ser + Gly165Ser + Trp208Thr  
Val103Ser + Gly135Glu + Leu221Cys  
Asn140Glu + Trp208Ala + Leu221Ser  
Gly 67Pro + Val103His + Ala164His  
Asn140Asp + Thr212Gln + Gly223Pro  
Gly139Gln + Thr195Gly + Pro214Asn  
Gly 69Pro + Leu104Glu + Ala114Pro  
Phe193Pro + Trp208Glu + Thr212Pro  
Gly136Gln + Trp208Cys + Leu221His  
Ser 70Asp + Thr 73Gly + Phe193Ser  
Tyr213Thr + Tyr218Gly + Gly223Asp  
Thr195Glu + Trp208Ser + Leu221Cys  
Asn163Asp + Leu221Ser + Gly223Pro  
Asn 66Gln + Gly139Asn + Ser220Asp  
Thr 73Ser + Pro214Asp + Leu221Ile  
Thr111Pro + Asn140Asp + Trp208Phe  
Asn166Glu + Pro214Asn + Thr217Ser  
Gly108Ser + Gly110Glu + Trp208Ala  
Leu134Asp + Gly165Asn + Tyr210Thr  
Phe193Thr + Thr217Gln + Ser220Asp  
Gly 67Pro + Trp112Gln + Ser192Asp  
Tyr210Ile + Thr217Gln + Ser222Asp  
Val103Ser + Leu104Ile + Ala164Asn  
Ala114Asp + Thr167Gly + Thr217Asn  
Thr111Asp + Thr195Gly + Tyr210Leu  
Tyr210Thr + Ser220Glu + Leu221Met  
Gly136Gln + Trp208Phe + Leu221Asp  
Thr113Ser + Trp208Val + Tyr210Thr  
Leu134Asn + Asn140Ser + Thr167Gln  
Gly136Ser + Trp208Val + Leu221Ala  
Gly165Pro + Pro214Ser + Ser220Glu  
Gly136Ser + Tyr210Leu + Thr217Gln  
Ser141Glu + Trp208Thr + Gly223Ser  
Asn 66Ser + Thr113Pro + Asn170Glu

Thr 73Pro + Tyr210Thr + Ser216Glu  
Thr 73Pro + Tyr210Glu + Leu221Ala  
Gly139Ser + Ala164Ser + Tyr210Gly  
Gly108Ser + Val138Ser + Ala164Gln  
Trp208Leu + Thr217Ser + Thr224Asp  
Asn 66Glu + Phe193Val + Tyr218Ala  
Gly108Asn + Tyr171Pro + Tyr210Thr  
Thr113Pro + Gly139Asn + Trp208Thr  
Asp105Glu + Asn166Gln + Tyr171Asn  
Ala114Pro + Thr217Gln + Ser220Asp  
Gly 67Glu + Ala114His + Thr217Asn  
Ser141Glu + Trp208Gln + Leu221Ser  
Gly 69Gln + Ala114Thr + Asn170Asp  
Asn163Asp + Ala219Thr + Leu221Ala  
Ser 70Asp + Thr217Gly + Ala219Thr  
Thr195Asn + Leu221Gly + Gly223Pro  
Gly 69Gln + Leu104Thr + Leu134Thr  
Gly139Asp + Gly165Pro + Leu221Gly  
Gly 67Asn + Gly108Glu + Tyr210Ile  
Val115Cys + Tyr210Val + Pro214Gly  
Leu104Met + Thr137Asp + Trp208Gly  
Trp112Gln + Leu134Ala + Ser192Asp  
Gly108Glu + Val115His + Ala219Gln  
Thr113Asn + Gly139Pro + Thr167Asp  
Thr137Asp + Trp208Gln + Gly223Asn  
Ser207Glu + Trp208Gln + Tyr210Asn  
Asn 66Ser + Ser207Glu + Tyr210Pro  
Val103Asn + Tyr210Ala + Thr215Glu  
Asn 66Ser + Trp208Phe + Ala219Thr  
Gly136Asn + Gly139Asn + Thr215Asn  
Ser109Glu + Asn166Ser + Thr217Asn  
Leu134Ile + Tyr171Gly + Thr217Gln  
Gly 72Gln + Gly110Asp + Gly135Asn  
Asn 68Glu + Asn106Gln + Thr113Ser  
Ala114Thr + Leu134Gln + Thr137Glu  
Ser107Asp + Gly139Ser + Tyr213Ser  
Thr113Asn + Thr167Gly + Leu221Met  
Gly 67Ser + Ser207Glu + Thr217Ser  
Asn 66Ser + Trp208Ile + Thr217Ser  
Trp208Ser + Leu221Gly + Ser222Glu  
Gly162Pro + Ser216Glu + Leu221Thr  
Leu104Cys + Asn106Gln + Ser207Glu  
Gly 69Gln + Pro169Gly + Tyr210Leu  
Thr 73Asp + Gly110Ser + Thr217Ser  
Gly 69Ser + Gly 72Asn + Thr215Gly  
Gly135Ser + Tyr171Ser + Ser191Glu  
Thr111Asn + Phe193Leu + Thr217Glu  
Trp208Ala + Leu221Ile + Thr224Asp  
Trp112Phe + Pro214Asp + Thr217Pro  
Asn 68Ser + Gly110Gln + Trp112Met  
Gly 69Ser + Leu104Cys + Trp208Asp  
Thr137Glu + Asn166Gln + Thr215Pro  
Gly108Glu + Tyr210Thr + Thr212Ser

Thr 73Gln + Gly162Ser + Tyr171Met  
Tyr171Val + Phe193Asp + Leu221Thr  
Gly 72Ser + Gly108Glu + Trp208Met  
Thr137Asn + Trp208Asp + Tyr210Gln  
Asn140Asp + Phe193Thr + Trp208Ala  
Gly 69Glu + Tyr210Ala + Tyr213Ser  
Asn 66Asp + Tyr218Ile + Leu221Cys  
Val103Thr + Leu134Ser + Asn163Gln  
Tyr171Gln + Ser192Glu + Gly223Ser  
Gly 69Asn + Thr137Glu + Leu221Pro  
Pro205Ser + Tyr218Glu + Leu221Asn  
Tyr210Met + Thr215Asp + Thr217Pro  
Thr 73Pro + Leu134Val + Gly136Glu  
Thr 73Ser + Ser107Glu + Trp208Ser  
Gly162Gln + Ser220Asp + Leu221Pro  
Val103Glu + Gly135Gln + Trp208Gly  
Asn140Asp + Tyr210Cys + Leu221His  
Gly108Asn + Ser192Asp + Tyr210His  
Asp105Glu + Trp208Asn + Tyr210Pro  
Thr 73Gln + Val103Pro + Trp112Tyr  
Leu134Cys + Asn170Ser + Tyr210Cys  
Asn106Ser + Ser109Glu + Leu221Cys  
Ser107Asp + Ala114Ser + Leu221Ala  
Gly 69Asn + Gly162Asn + Leu221Asn  
Gly 67Ser + Gly135Asp + Gly162Asn  
Val115Gly + Leu134Ser + Ala164Gln  
Asn 66Glu + Leu104Asn + Ala168Gly  
Gly 69Ser + Asn166Glu + Tyr218Asn  
Thr113Asp + Ala204Ser + Tyr218Ser  
Asn166Gln + Trp208Thr + Thr217Asp  
Gly108Glu + Thr217Ser + Tyr218Gly  
Asn106Asp + Trp208Met + Thr217Gln  
Gly 69Glu + Tyr171Val + Trp208His  
Gly139Asn + Pro169Gly + Trp208Asn  
Val115Ala + Thr215Gln + Leu221Glu  
Thr111Pro + Pro169Gln + Ala219Glu  
Thr 73Glu + Thr137Asn + Trp208His  
Asn 66Asp + Asn106Ser + Leu221Thr  
Gly 67Pro + Gly 69Ser + Thr224Ser  
Val115His + Ser191Asp + Tyr218Val  
Thr137Glu + Thr195Asn + Trp208Gly  
Leu134Glu + Trp208Ser + Leu221Ser  
Val103Ser + Trp208Glu + Thr217Asn  
Gly139Asn + Phe193Pro + Leu221Cys  
Asn106Gln + Ala164Pro + Asn166Asp  
Asp105Glu + Val138Ser + Thr217Pro  
Gly135Pro + Tyr171Thr + Ser222Glu  
Ser216Asp + Thr217Asn + Thr224Gly  
Ala114Gly + Asn140Gln + Tyr218Asp  
Ser107Asp + Pro214Gly + Leu221Ser  
Asp105Glu + Asn170Ser + Thr195Pro  
Ser 70Glu + Tyr171Leu + Pro214Ser  
Gly 67Asp + Leu104Thr + Gly223Asn

Gly108Asp + Ala114Asn + Tyr210Cys  
Gly110Asn + Pro214Gly + Ser220Asp  
Gly 69Asn + Pro169Gln + Ser220Glu  
Gly162Glu + Trp208Ala + Thr217Asn  
Trp112Tyr + Val115Pro + Ser220Glu  
Trp208Gly + Tyr210Ile + Pro214Asp  
Leu134Asn + Pro169Ser + Leu221Asp  
Thr113Ser + Trp208Leu + Leu221Glu  
Asn166Ser + Tyr218Ile + Ser220Glu  
Trp112Pro + Phe193His + Pro214Asp  
Leu104Glu + Val115Ser + Ala164Ser  
Gly 69Pro + Pro169Asp + Leu221His  
Gly108Gln + Val138Ser + Ser220Glu  
Gly110Asn + Thr137Gln + Ser220Glu  
Ala164Thr + Asn166Ser + Tyr210Asp  
Trp112Pro + Val115Pro + Pro214Glu  
Val115Glu + Gly139Asn + Tyr210Gln  
Gly 69Ser + Thr 73Ser + Tyr210Pro  
Ala114Ser + Ser207Asp + Thr224Pro  
Thr 73Pro + Asn166Glu + Thr212Gly  
Gly 72Gln + Gly110Asp + Leu221His  
Tyr210Cys + Pro214Asn + Tyr218Asp  
Gly 69Asn + Gly136Pro + Asn166Ser  
Thr 73Gly + Gly108Ser + Ser220Glu  
Thr 73Gly + Pro205Ser + Thr224Ser  
Val103Met + Ala164Asp + Leu221Met  
Thr111Asn + Thr167Glu + Phe193Met  
Thr 73Asp + Tyr210Asn + Thr217Gln  
Asn 66Glu + Thr 73Gly + Leu221Gln  
Thr113Asp + Asn166Ser + Thr167Pro  
Asn 66Gln + Tyr171Gly + Tyr210Pro  
Asn 68Ser + Gly 69Pro + Asn170Gln  
Asn 68Ser + Val115Glu + Tyr210Asn  
Thr113Gln + Ser191Asp + Leu221His  
Thr167Pro + Trp208Val + Tyr210Leu  
Gly162Glu + Trp208Ala + Thr217Pro  
Leu104Met + Ser207Asp + Thr212Gly  
Thr137Gln + Asn163Asp + Pro205Gln  
Val115Thr + Thr217Gln + Ser220Asp  
Ser 70Asp + Val138Ser + Tyr210Val  
Pro214Gly + Leu221Ile + Thr224Asp  
Leu134Val + Gly165Gln + Trp208Thr  
Ser109Asp + Ala114Asn + Tyr210Ser  
Asn 66Ser + Val115Asn + Trp208Gln  
Thr 73Pro + Phe193Glu + Gly206Gln  
Leu134Ser + Leu221Pro + Ser222Asp  
Val115Cys + Ala164Glu + Asn170Gln  
Ala164Asn + Trp208Cys + Thr217Glu  
Asn 66Glu + Ala168Pro + Thr215Pro  
Gly135Ser + Ser207Asp + Thr217Gln  
Asn140Gln + Thr167Asp + Trp208Met  
Gly165Gln + Trp208Thr + Leu221Asn  
Thr 73Pro + Asn106Glu + Leu221Cys

Thr113Ser + Leu221Ile + Gly223Asp  
Asn 66Asp + Gly136Gln + Gly162Gln  
Ser191Glu + Thr217Asn + Thr224Pro  
Leu104Gln + Gly162Glu + Tyr210Gln  
Trp112Gln + Asn163Asp + Thr217Gly  
Leu134Pro + Thr217Asn + Leu221Glu  
Gly165Pro + Tyr210Met + Thr217Glu  
Ala164Gly + Ser191Glu + Trp208Cys  
Val115Cys + Gly139Asn + Pro205Asn  
Gly135Pro + Trp208Ala + Tyr210Pro  
Pro169Asn + Thr195Glu + Tyr210His  
Ser107Asp + Trp208Met + Tyr210Pro  
Thr 73Gly + Ala164Gly + Tyr210Glu  
Leu104Val + Pro205Gln + Leu221Asp  
Thr111Gln + Ser141Asp + Leu221Asn  
Trp112Cys + Thr167Gln + Thr217Pro  
Val138Thr + Asn170Glu + Leu221Pro  
Ser 70Asp + Leu134Ser + Thr224Asn  
Thr137Asn + Ser207Asp + Tyr218Thr  
Gly135Glu + Ala164Asn + Ala203Ser  
Leu134Gly + Trp208Glu + Thr212Ser  
Asn163Asp + Trp208Asn + Tyr210Thr  
Val115Thr + Ser192Glu + Thr217Ser  
Gly 72Pro + Ser109Asp + Tyr210Gln  
Ser109Glu + Asn163Gln + Phe193Pro  
Gly136Pro + Pro169Glu + Leu221Ser  
Gly110Asn + Ser220Glu + Leu221Met  
Trp112Gly + Tyr210Asp + Thr217Pro  
Trp208Ser + Tyr210Ser + Leu221Pro  
Trp112Met + Ser192Glu + Leu221Ala  
Thr137Gly + Thr167Ser + Thr217Glu  
Ser109Asp + Gly162Pro + Tyr210Ala  
Ala114Thr + Gly162Asn + Ser191Glu  
Thr195Asn + Tyr218Pro + Leu221Glu  
Thr 73Glu + Ala164Gln + Pro214Ser  
Asn 68Glu + Trp208Tyr + Thr217Asn  
Gly165Pro + Thr212Ser + Ser220Glu  
Gly 67Asn + Gly139Gln + Ala168Asp  
Ser141Glu + Pro214Gln + Leu221Gln  
Gly206Ser + Trp208Tyr + Thr215Gln  
Leu134Pro + Gly162Asn + Phe193Ile  
Trp112Ala + Thr217Asn + Ser220Asp  
Thr111Gly + Gly139Glu + Thr212Asn  
Ser107Glu + Tyr213Ala + Thr217Pro  
Gly165Ser + Thr217Glu + Gly223Pro  
Tyr210His + Thr217Asp + Leu221Ala  
Trp208Cys + Thr217Asp + Tyr218Asn  
Gly 69Gln + Val103Ala + Ser220Glu  
Asn106Ser + Tyr210Gly + Gly223Ser  
Trp208Cys + Tyr210Asn + Thr217Asp  
Asn 66Gln + Gly139Gln + Thr217Gly  
Thr 73Glu + Leu104Ile + Tyr210Ile  
Thr113Ser + Pro169Asn + Thr217Ser

Tyr210Gly + Pro214Gly + Leu221Glu  
Leu134Ile + Ala219Glu + Ser220Asp  
Asn140Asp + Ser141Glu + Tyr218Ser  
Tyr213Val + Ser220Glu + Leu221Glu  
Tyr210Val + Ser220Glu + Leu221Asp  
Ala164His + Ser220Glu + Leu221Glu  
Tyr171His + Ser220Asp + Leu221Glu  
Tyr210Ile + Ser220Glu + Leu221Asp  
Trp112Leu + Ser220Asp + Leu221Glu  
Gly136Pro + Ser191Asp + Ser192Glu  
Ala168Ser + Thr217Asp + Tyr218Glu  
Asn166Ser + Thr217Glu + Tyr218Asp  
Gly162Asp + Asn163Glu + Leu221Met  
Trp208Ser + Tyr210Glu + Ser220Asp  
Ala114Thr + Gly165Asp + Phe193Glu  
Ser207Glu + Thr217Asn + Ser222Glu  
Asn140Ser + Trp208Glu + Ser220Asp  
Trp208Asp + Leu221Asn + Ser222Glu  
Gly139Gln + Trp208Glu + Leu221Asp  
Asn 68Glu + Asp105Glu + Asn106Asp  
Thr137Gln + Gly139Asp + Tyr171Glu  
Trp208Asp + Tyr210Glu + Leu221Ala  
Gly 69Pro + Asn166Glu + Ala168Glu  
Tyr171Cys + Tyr210Glu + Leu221Asp  
Thr137Glu + Pro169Asp + Asn170Ser  
Gly162Glu + Tyr171Asp + Thr217Ser  
Leu134Ile + Ser191Glu + Gly223Glu  
Val115Pro + Ser207Glu + Ser220Asp  
Gly139Pro + Ser207Asp + Ser220Glu  
Ser 70Glu + Val103Pro + Asn106Asp  
Gly 69Asp + Thr 73Asp + Tyr218Ile  
Asn 66Asp + Trp208Gly + Pro214Glu  
Asn 66Asp + Ser 70Glu + Gly136Ser  
Asn163Asp + Ser191Asp + Ser207Glu  
Asn 66Ser + Gly 69Asp + Ser109Glu  
Gly 67Glu + Trp208Ile + Thr215Asp  
Ser 70Glu + Tyr210Gln + Thr217Glu  
Ser 70Glu + Gly135Pro + Thr217Glu  
Val138Glu + Ser141Asp + Tyr210Gln  
Val138Glu + Ser141Glu + Pro214Asn  
Ser107Asp + Gly110Asp + Ala114Glu  
Ser109Glu + Ala114Glu + Gly136Ser  
Gly110Glu + Gly136Glu + Tyr210Gln  
Gly135Ser + Tyr210Asp + Thr217Glu  
Ser 70Glu + Pro169Asn + Leu221Asp  
Val115Gly + Gly162Asp + Thr167Asp  
Gly110Pro + Thr217Asp + Ser220Asp  
Ala164His + Thr217Asp + Ser220Asp  
Tyr210Cys + Thr217Asp + Ser220Asp  
Val103Ser + Thr217Asp + Ser220Asp  
Thr217Glu + Tyr218Val + Ser220Glu  
Gly 69Ser + Thr217Glu + Ser220Glu  
Tyr210Val + Thr217Asp + Ser220Glu



Gly 67Glu + Ser107Glu + Thr111Asn  
 Asn166Asp + Ser191Asp + Leu221Val  
 Leu134Asp + Phe193Glu + Gly223Asp  
 Asn 66Asp + Ser216Asp + Thr217Glu  
 Val103Glu + Asn106Glu + Thr113Glu  
 Ser 70Asp + Gly139Ser + Ser220Asp  
 Leu104Asp + Asp105Glu + Ser191Glu  
 Ala114Glu + Ser216Asp + Thr217Glu  
 Thr195Glu + Ser216Asp + Thr217Glu  
 Val115Glu + Ser220Asp + Leu221Glu  
 Ser109Asp + Ser220Asp + Leu221Glu  
 Thr215Glu + Ser216Asp + Ser220Asp  
 Ser192Asp + Phe193Glu + Thr217Asp  
 Gly165Asp + Tyr210Glu + Ser220Asp  
 Ser 70Glu + Asp105Glu + Tyr218Asp  
 Gly 69Glu + Ala168Glu + Thr195Glu

TABLE 34

## Multi-loop Quadruple Mutation Variants

Asn 66Gln + Leu104Gly + Tyr210Pro + Thr217Glu  
 Gly 67Ser + Gly 69Ser + Val103Gln + Gly223Asp  
 Trp112Cys + Ala164Glu + Trp208Gln + Thr217Gly  
 Leu134Ile + Trp208Gln + Ala219Gly + Ser220Glu  
 Leu104Ala + Ser109Glu + Thr111Gln + Val115Ala  
 Gly108Glu + Ala164Gly + Gly165Gln + Thr167Pro  
 Gly 69Glu + Asn106Gln + Trp112Cys + Thr113Pro  
 Thr 73Asp + Leu134Ser + Thr137Asn + Thr217Pro  
 Gly 67Ser + Gly136Pro + Thr195Ser + Leu221Ala  
 Thr 73Gly + Leu104Gly + Trp208Pro + Thr215Gly  
 Gly110Glu + Gly136Pro + Phe193Met + Thr217Pro  
 Ala164Gln + Tyr171Ile + Phe193Ile + Thr224Gly  
 Gly136Pro + Ala164Thr + Ser207Asp + Trp208Leu  
 Gly108Gln + Trp112Pro + Trp208Thr + Tyr210Glu  
 Thr111Asp + Leu134Val + Tyr210His + Thr217Pro  
 Gly136Gln + Trp208Phe + Ala219Asp + Leu221Met  
 Thr113Ser + Tyr171Leu + Trp208Val + Tyr210Thr  
 Gly136Ser + Ser141Glu + Trp208Thr + Tyr210Leu  
 Asn 66Ser + Thr 73Pro + Thr113Pro + Asn170Glu  
 Thr 73Pro + Gly139Ser + Tyr210Glu + Leu221Ala  
 Gly108Ser + Val138Ser + Trp208Leu + Thr217Ser  
 Asn 68Ser + Gly110Gln + Trp112Met + Ser192Asp  
 Thr 73Gln + Val103Ala + Gly162Ser + Ala164Glu  
 Thr137Asn + Asn140Asp + Phe193Thr + Trp208Ala  
 Leu134Ser + Asn163Gln + Ser220Glu + Gly223Asn  
 Asn140Glu + Tyr171Gln + Tyr210Val + Gly223Ser  
 Thr 73Pro + Leu134Val + Gly136Glu + Thr217Pro  
 Ser107Glu + Gly162Gln + Trp208Ser + Leu221Pro  
 Gly108Asn + Gly162Asn + Ser192Asp + Tyr210Cys  
 Val103Pro + Ser109Glu + Trp208Asn + Tyr210Pro  
 Thr 73Gln + Trp112Tyr + Leu134Cys + Asn170Ser  
 Gly 69Asn + Gly162Asn + Asn166Ser + Leu221Asn

Gly 67Ser + Leu134Ser + Gly162Asn + Ala164Gln  
Leu134Ile + Gly135Pro + Ala168Thr + Trp208Met  
Trp112Ser + Ala168His + Thr217Ser + Leu221Ala  
Gly110Ser + Thr111Gln + Thr215Ser + Ser222Asp  
Thr111Pro + Thr137Asn + Pro169Gln + Ala219Glu  
Thr 73Glu + Asn106Ser + Trp208His + Leu221Thr  
Asn 66Asp + Gly 67Pro + Gly 69Ser + Thr224Ser  
Leu104Cys + Val115His + Ser191Asp + Tyr218Val  
Leu134Glu + Thr195Asn + Trp208Ser + Leu221Ser  
Val103Ser + Gly139Asn + Thr217Asn + Leu221Cys  
Asn106Gln + Ala164Pro + Asn166Asp + Phe193Pro  
Asp105Glu + Gly135Pro + Val138Ser + Thr217Pro  
Gly 67Asp + Thr113Asn + Ala114Asn + Tyr210Cys  
Asn166Asp + Thr195Gly + Tyr210Gly + Thr224Gln  
Trp112Pro + Asn166Ser + Phe193His + Tyr218Ile  
Gly 69Pro + Leu104Glu + Val115Ser + Ala164Ser  
Trp112Pro + Val115Glu + Gly139Asn + Tyr210Gln  
Gly 69Ser + Thr 73Ser + Tyr210Pro + Thr224Pro  
Gly 72Gln + Thr 73Pro + Gly110Asp + Leu221His  
Thr111Asn + Gly136Pro + Tyr210Thr + Tyr218Asp  
Gly108Asp + Gly136Asn + Asn166Ser + Leu221Thr  
Val103Met + Pro205Ser + Leu221Met + Thr224Ser  
Gly 69Asn + Thr 73Asp + Trp208Gln + Tyr210Leu  
Thr 73Gly + Asn166Ser + Thr167Pro + Leu221Gln  
Asn 66Gln + Thr113Asp + Tyr171Gly + Tyr210Pro  
Gly162Glu + Thr167Pro + Trp208Val + Tyr210Leu  
Ser207Asp + Trp208Ala + Thr212Gly + Thr217Pro  
Leu104Met + Thr137Gln + Asn163Asp + Pro205Gln  
Ser109Asp + Leu134Val + Gly165Gln + Trp208Thr  
Ala114Asn + Val115Asn + Trp208Gln + Tyr210Ser  
Asn 66Glu + Ala164Asn + Trp208Cys + Thr215Pro  
Gly135Ser + Ala168Pro + Ser207Asp + Thr217Gln  
Thr167Asp + Trp208Met + Thr217Gln + Leu221Asn  
Thr 73Pro + Asn106Glu + Thr113Ser + Leu221Cys  
Gly162Gln + Ser191Glu + Thr217Asn + Thr224Pro  
Gly 69Gln + Leu104Gln + Gly162Glu + Tyr210Gln  
Leu104Gln + Tyr171Pro + Thr217Gly + Leu221Glu  
Gly110Glu + Phe193Asn + Trp208Cys + Gly223Pro  
Ala164Gly + Ser191Glu + Pro205Asn + Trp208Cys  
Gly135Pro + Ser207Glu + Trp208Ala + Tyr210Cys  
Thr 73Glu + Leu104Val + Pro205Gln + Leu221Cys  
Trp112Cys + Val138Thr + Thr167Gln + Thr217Pro  
Ser109Asp + Ala164His + Gly223Pro + Thr224Asn  
Asn 68Gln + Tyr210Pro + Thr212Ser + Ser220Asp  
Leu134Gly + Trp208Glu + Tyr210Met + Thr212Ser  
Asn163Asp + Asn170Gln + Trp208Asn + Tyr210Thr  
Trp112Gly + Trp208Ser + Tyr210Asp + Thr217Pro  
Trp112Met + Ser192Glu + Trp208Ser + Tyr210Val  
Trp112Leu + Ser220Asp + Leu221Ala + Thr224Asn  
Leu104Met + Thr111Asp + Ala164Gln + Pro214Ser  
Gly139Gln + Gly165Pro + Thr212Ser + Ser220Glu  
Thr113Asp + Leu134His + Trp208Ile + Leu221Ala  
Asp105Glu + Pro214Gln + Thr215Gln + Leu221Gln

Leu134Pro + Gly162Asn + Gly206Ser + Trp208Tyr  
Trp112Ala + Phe193Ile + Thr217Asn + Ser220Asp  
Ser107Glu + Thr212Asn + Tyr213Ala + Thr217Pro  
Gly 67Gln + Gly 69Gln + Thr113Asn + Ser220Glu  
Asn 66Gln + Trp208Cys + Tyr210Asn + Thr217Asp  
Thr 73Glu + Leu104Ile + Gly139Gln + Thr217Gly  
Thr113Ser + Pro169Asn + Tyr210Ile + Thr217Ser  
Leu134Pro + Thr167Gln + Tyr210Met + Ser220Asp  
Thr 73Glu + Asn166Gln + Tyr171His + Leu221Ala  
Val138Met + Asn170Gln + Thr217Asn + Ala219His  
Gly136Gln + Ala219Ser + Ser220Asp + Thr224Gln  
Gly 69Asn + Asn166Gln + Tyr210Glu + Thr217Gly  
Thr 73Ser + Gly165Gln + Thr212Pro + Ser222Glu  
Thr137Pro + Asn140Gln + Tyr171Gly + Ser220Glu  
Tyr210Pro + Tyr213Ile + Ser220Asp + Leu221Pro  
Val103Asp + Asn106Ser + Phe193His + Thr217Gly  
Asn 68Asp + Thr113Asn + Val138Ala + Trp208His  
Asp105Glu + Leu134Met + Gly165Ser + Leu221Gln  
Asn 68Gln + Trp208Thr + Thr217Ser + Ser220Asp  
Tyr171Asn + Thr195Asn + Tyr210Asn + Tyr218Asn  
Asn 66Ser + Pro169Ser + Tyr210Cys + Gly223Glu  
Ala114Gln + Phe193Glu + Tyr210Gly + Thr224Pro  
Asn 66Ser + Gly136Ser + Ala164Asp + Ala168Gln  
Leu104Gly + Gly162Pro + Ser192Asp + Leu221Asn  
Gly108Asp + Gly162Pro + Trp208Thr + Gly223Asn  
Asn 66Glu + Thr113Gly + Ala164Pro + Gly223Pro  
Gly110Pro + Trp112Ala + Ser216Glu + Leu221His  
Val115Met + Pro169Ser + Trp208His + Tyr210Asp  
Leu104Ser + Ser109Asp + Gly110Pro + Thr195Gly  
Leu104Asn + Thr137Glu + Trp208Ser + Leu221Val  
Asn106Gln + Ala114Asp + Tyr171Thr + Leu221Pro  
Pro205Asn + Tyr210Asn + Thr217Gly + Tyr218Leu  
Leu104Pro + Asn106Ser + Thr137Asp + Thr217Pro  
Gly110Ser + Thr137Gln + Asn170Glu + Thr217Asn  
Thr113Gly + Phe193Met + Trp208His + Tyr213Ile  
Asn166Gln + Ser207Asp + Thr217Ser + Tyr218Met  
Gly162Asp + Phe193Pro + Trp208Tyr + Thr217Gln  
Gly 69Ser + Ser107Glu + Trp208Met + Tyr210Leu  
Val103Asp + Thr167Pro + Ile209Val + Tyr210Gln  
Thr111Asn + Asn170Ser + Trp208Tyr + Thr212Gly  
Ser107Glu + Thr137Pro + Gly162Ser + Trp208Cys  
Val103Thr + Ala164Thr + Ala168Glu + Thr217Ser  
Gly139Pro + Tyr210Asn + Leu221Pro + Thr224Pro  
Leu134Ser + Ser141Glu + Tyr210Ala + Thr217Gln  
Val103Asn + Leu134Ala + Tyr218Met + Thr224Asn  
Asn 66Asp + Gly135Gln + Asn163Ser + Leu221Val  
Gly 69Glu + Val115Pro + Thr137Pro + Trp208Ile  
Gly 69Pro + Gly165Pro + Tyr210Ser + Leu221Ile  
Asn 66Gln + Gly110Glu + Trp112Gly + Trp208Pro  
Trp112Ala + Ala114Pro + Gly135Ser + Asn163Asp  
Gly 67Asn + Ser207Glu + Tyr210Thr + Thr217Pro  
Asn166Asp + Thr167Pro + Ile209His + Tyr210Val  
Thr 73Ser + Asn106Gln + Tyr171Glu + Thr217Asn

Vall115Glu + Tyr210Ser + Thr217Gln + Ala219Gly  
Leu104Pro + Thr195Gln + Trp208Tyr + Ser222Asp  
Ser141Asp + Ala204Gln + Trp208Val + Tyr210Leu  
Gly 67Ser + Gly 69Glu + Vall103Gly + Tyr210Gln  
Tyr171Met + Phe193Leu + Thr215Asp + Thr217Gln  
Asn 68Gln + Asp105Glu + Trp208Phe + Leu221Ala  
Leu104Asn + Vall115Gln + Leu134Gln + Pro214Glu  
Gly165Ser + Ser191Asp + Pro214Gly + Leu221Thr  
Gly 67Ser + Asn 68Gln + Tyr210Val + Ser220Asp  
Ser109Asp + Vall138Gly + Trp208Ser + Thr217Pro  
Thr111Gly + Gly136Pro + Ser141Asp + Leu221Ile  
Trp112Asn + Gly162Ser + Ile209Cys + Thr224Gln  
Vall115Ala + Pro169Glu + Tyr210Val + Leu221Cys  
Gly135Ser + Tyr210Leu + Thr217Glu + Leu221Pro  
Asn 66Gln + Ser109Asp + Gly110Gln + Tyr218Thr  
Thr 73Gly + Trp208Ile + Thr217Glu + Leu221Asn  
Leu134Ser + Trp208His + Tyr210Leu + Ser220Glu  
Gly135Pro + Thr137Gln + Ala219Ser + Leu221Gln  
Asn 68Gln + Asn106Glu + Pro169Gln + Thr215Gln  
Vall138Gln + Gly165Glu + Tyr218Leu + Leu221Cys  
Phe193Gln + Ser207Asp + Trp208Cys + Thr217Gln  
Gly 67Asn + Gly165Asn + Phe193Tyr + Pro205Ser  
Asn 66Ser + Ala168Ser + Tyr210Cys + Thr217Asp  
Pro214Gly + Ser216Asp + Thr217Gln + Leu221His  
Asn 66Gln + Vall115Gln + Asn166Asp + Tyr218Met  
Thr111Glu + Gly165Ser + Ala219His + Leu221Thr  
Ser141Asp + Asn163Ser + Phe193Tyr + Ala204Ser  
Gly108Pro + Asn163Ser + Gly165Asp + Tyr213Ser  
Trp208His + Tyr210Thr + Tyr218Gln + Ser220Asp  
Gly 69Glu + Ala164Pro + Trp208Val + Thr224Ser  
Ser109Asp + Ala203Pro + Trp208His + Thr217Ser  
Thr 73Asp + Tyr171Cys + Tyr210Pro + Leu221Pro  
Gly162Pro + Tyr218Val + Ala219Thr + Ser220Glu  
Asn170Gln + Ser192Glu + Trp208Pro + Tyr210Gly  
Thr113Ser + Thr137Gln + Phe193Ser + Pro205Ser  
Trp112Pro + Thr137Gly + Ser220Asp + Leu221Asn  
Gly162Glu + Ala164Ser + Ala168His + Pro214Asn  
Ala114Pro + Leu134Glu + Asn140Ser + Phe193Ile  
Ala164Gln + Ala168Glu + Trp208Asn + Tyr210Leu  
Vall103Pro + Gly139Pro + Asn163Asp + Gly165Gln  
Gly162Ser + Trp208Glu + Tyr210Ser + Leu221Gln  
Ser141Asp + Tyr171His + Trp208Ala + Leu221Met  
Asn 66Ser + Gly139Gln + Asn163Gln + Tyr210Thr  
Leu134Asp + Gly136Ser + Asn163Gln + Trp208Val  
Ser191Asp + Phe193Leu + Pro214Gln + Thr217Ser  
Gly 67Asn + Gly108Asp + Thr195Asn + Thr215Pro  
Ala114Thr + Asn140Glu + Asn166Ser + Ala203Pro  
Ser191Glu + Ala203Gly + Trp208Asn + Tyr210Ala  
Asn140Asp + Ser141Glu + Ala164Gln + Tyr218Ser  
Thr 73Ser + Tyr213Val + Ser220Glu + Leu221Glu  
Phe193Met + Thr215Gln + Ser220Glu + Leu221Glu  
Thr113Ser + Trp208Leu + Ser220Glu + Leu221Glu  
Thr 73Pro + Vall115Ala + Ser192Asp + Phe193Glu

Ala114Asn + Trp208Pro + Tyr210Glu + Ser220Glu  
Trp208Cys + Tyr210Glu + Ala219Asn + Ser220Asp  
Ala114Pro + Gly139Pro + Tyr210Asp + Ser220Glu  
Tyr210Glu + Thr217Gln + Tyr218Gln + Ser220Glu  
Asn140Ser + Trp208Glu + Ser220Asp + Leu221Asp  
Val103Pro + Trp208Glu + Tyr210Ser + Ser220Glu  
Thr 73Gln + Ser107Asp + Ser109Glu + Asn163Gln  
Gly 67Gln + Ser107Asp + Ser109Glu + Leu221Cys  
Asp105Glu + Asn106Ser + Ser109Glu + Thr217Pro  
Leu104Glu + Asp105Glu + Ser107Asp + Leu221Ser  
Gly206Pro + Pro214Asp + Thr217Glu + Leu221Asn  
Ser207Asp + Trp208Glu + Ser220Asp + Leu221Ser  
Gly135Asp + Gly162Glu + Tyr171Asp + Thr217Ser  
Gly 69Glu + Leu104Asp + Asp105Glu + Tyr210Ala  
Ala168Asn + Ser220Asp + Leu221Pro + Ser222Asp  
Phe193Gln + Ser207Glu + Thr217Ser + Leu221Glu  
Gly110Gln + Gly165Gln + Ser222Asp + Thr224Asp  
Asn106Glu + Ser109Asp + Phe193Ile + Trp208Leu  
Gly110Asn + Thr137Glu + Pro169Asp + Asn170Ser  
Gly136Glu + Gly162Glu + Trp208Ala + Thr217Asn  
Leu134Ile + Ser191Glu + Leu221His + Gly223Glu  
Gly139Pro + Tyr171Met + Ser207Asp + Ser220Glu  
Val103Pro + Thr111Gly + Ser207Asp + Ser220Asp  
Gly108Asn + Thr195Gly + Ser207Asp + Ser220Asp  
Ser 70Glu + Val103Pro + Asn106Asp + Ala168Pro  
Thr113Gly + Gly162Ser + Asn163Asp + Asn170Glu  
Leu104Ile + Ser107Glu + Gly110Asp + Asn170Gln  
Thr 73Gly + Gly135Asp + Pro169Asp + Trp208Thr  
Tyr210Met + Thr215Asp + Tyr218Glu + Leu221Asn  
Leu104Thr + Gly136Glu + Pro169Asp + Leu221Val  
Asn 66Asp + Thr 73Glu + Tyr218Ile + Leu221Cys  
Asp105Glu + Thr111Asp + Ala114Pro + Val138Met  
Asn 68Glu + Thr 73Glu + Trp208Tyr + Thr217Asn  
Ser207Glu + Tyr213Ile + Ala219Asp + Ser222Asp  
Asn163Asp + Ala164Ser + Ser191Asp + Ser207Glu  
Thr 73Glu + Ala168Ser + Tyr210Glu + Tyr218Glu  
Gly 67Glu + Trp208Ile + Pro214Asn + Thr215Asp  
Asp105Glu + Thr111Glu + Thr113Glu + Asn163Gln  
Ser 70Glu + Tyr210His + Thr217Asp + Leu221Ala  
Gly 67Glu + Gly108Asp + Thr137Gln + Thr167Pro  
Ser107Asp + Gly110Asp + Ala114Glu + Tyr218Asn  
Ser 70Asp + Tyr210Glu + Thr217Asn + Ser220Glu  
Thr113Gly + Trp208Glu + Ser220Asp + Thr224Asp  
Val138Met + Ala219Glu + Ser222Asp + Thr224Gln  
Ser 70Glu + Trp112Tyr + Tyr218Cys + Leu221Glu  
Gly 67Ser + Ser 70Glu + Pro169Asn + Leu221Asp  
Val115Cys + Gly139Asn + Thr217Asp + Ser220Asp  
Gly 69Ser + Val115Thr + Thr217Glu + Ser220Glu  
Ala168His + Trp208Tyr + Thr217Asp + Ser220Glu  
Thr111Gln + Gly135Pro + Thr217Asp + Ser220Glu  
Ala164Thr + Ser191Glu + Trp208Glu + Thr215Asn  
Asn 68Glu + Val103Glu + Ser109Glu + Asn166Gln  
Ser 70Asp + Gly139Ser + Thr217Glu + Ser220Asp

Leu134Asp + Phe193Glu + Ala203Ser + Gly223Asp  
Asn 66Asp + Asn 68Ser + Ser216Asp + Thr217Glu  
Ala164Glu + Trp208Cys + Leu221Glu + Ser222Asp  
Ser 70Glu + Ser207Glu + Ala219Ser + Ser220Glu  
Gly 67Asp + Ser 70Asp + Thr 73Gly + Tyr210Glu  
Trp208Ile + Thr217Glu + Tyr218Asp + Ser222Asp  
Leu104Ile + Thr111Glu + Gly135Asp + Asn163Gln  
Ala114Glu + Leu134Ile + Ala219Glu + Ser220Asp  
Asn166Glu + Thr167Gly + Ser222Asp + Gly223Asp  
Gly139Glu + Tyr210Ala + Ser222Asp + Gly223Glu  
Val103Gly + Gly110Glu + Thr111Glu + Ser222Glu  
Ala114Glu + Asn170Ser + Ser216Asp + Thr217Glu  
Gly108Asp + Ser109Asp + Tyr210Gln + Thr217Glu  
Asn 68Ser + Ser109Asp + Gly110Glu + Ser220Glu  
Asn 68Glu + Asn163Ser + Ser207Asp + Trp208Glu  
Ser207Asp + Trp208Glu + Thr217Glu + Leu221Cys  
Gly 69Pro + Asp105Glu + Asn106Asp + Asn166Asp  
Ser109Asp + Tyr210Gln + Ser220Glu + Leu221Asp  
Tyr171Gly + Ser192Glu + Ser220Asp + Leu221Glu  
Asn170Glu + Ser191Glu + Ser192Glu + Pro205Ser  
Asn 66Asp + Tyr210Gln + Thr217Glu + Tyr218Glu  
Asn106Asp + Ser107Glu + Thr137Asp + Tyr210Gln  
Ser192Asp + Phe193Glu + Pro205Ser + Thr217Asp  
Ser 70Asp + Gly162Asp + Asn163Glu + Leu221Ser  
Gly 69Asn + Asn106Glu + Tyr210Asp + Ser220Glu  
Val103Ser + Asn140Asp + Tyr210Glu + Ser220Asp  
Gly 69Glu + Phe193His + Ser207Glu + Ser222Asp  
Gly162Glu + Asn163Gln + Trp208Asp + Ser220Glu  
Val115Cys + Asn170Asp + Trp208Asp + Ser220Asp  
Gly110Glu + Trp208Asp + Leu221Asn + Ser222Glu  
Pro169Asp + Tyr171Glu + Pro214Asn + Gly223Glu  
Asn163Glu + Ser207Glu + Trp208Met + Tyr210Thr  
Gly 67Glu + Ser 70Glu + Val115Cys + Gly162Glu  
Gly162Glu + Ala164Glu + Ser220Glu + Leu221Gly  
Asn 66Glu + Val103Asp + Gly108Asn + Leu221Met  
Asp105Glu + Ser109Glu + Tyr218Asp + Leu221Ala  
Gly 67Asp + Ala164Asp + Ala168Glu + Tyr213Asn  
Gly 69Pro + Ser141Asp + Pro214Asp + Thr217Asp  
Thr 73Glu + Trp112Ile + Trp208Gly + Ser220Asp  
Gly 72Ser + Ser107Asp + Thr111Asp + Asn166Gln  
Gly165Glu + Ser192Asp + Thr217Asp + Leu221Ile  
Gly135Glu + Asn140Glu + Ala204His + Pro214Ser  
Asp105Glu + Gly110Asp + Asn170Asp + Ala219Gly  
Asn 66Asp + Asn 68Asp + Leu134Gly + Asn163Glu  
Leu104Thr + Ala168Asp + Asn170Asp + Ser220Glu  
Thr 73Asp + Ser109Glu + Pro214Glu + Thr217Asn  
Thr 73Asp + Val115Cys + Pro214Asp + Thr224Glu  
Ser109Asp + Thr113Asp + Leu134Asn + Phe193Pro  
Gly 69Ser + Gly108Asp + Gly110Asp + Ser220Glu  
Gly139Asp + Ser141Asp + Ala164Asp + Leu221Val  
Asn 66Asp + Trp208Met + Ser220Asp + Ser222Glu  
Trp112Asp + Tyr210Thr + Ser220Glu + Ser222Glu  
Ser109Asp + Ser207Glu + Tyr210Gly + Leu221Glu

Gly 69Asp + Asp105Glu + Thr137Asp + Trp208Asn  
Ser109Asp + Thr111Glu + Ser191Glu + Tyr210Leu  
Gly139Gln + Pro169Asp + Ser191Asp + Ser207Glu  
Gly 67Ser + Asn140Asp + Ser222Asp + Thr224Glu  
Val138Asp + Asn170Glu + Trp208His + Thr215Glu  
Asn 68Asp + Thr111Gly + Val138Asp + Asn170Asp  
Leu104Glu + Ser107Asp + Gly135Gln + Tyr171Asp  
Leu104Pro + Trp112Asp + Asn140Glu + Ser222Glu  
Gly139Glu + Asn170Asp + Gly206Pro + Ser220Glu  
Ala164Asp + Asn170Asp + Ser216Asp + Thr217Pro  
Thr113Glu + Ala114His + Ser207Glu + Ser220Glu  
Gly110Glu + Val115Gln + Ser207Asp + Thr224Glu  
Asn106Glu + Asn163Glu + Asn170Glu + Leu221His  
Gly 69Asp + Thr 73Glu + Thr113Asn + Ser141Glu  
Gly 69Asp + Thr 73Asp + Thr111Gly + Gly139Glu  
Ser107Asp + Asn163Glu + Ser192Asp + Trp208Met  
Asn 68Asp + Gly108Asp + Ser207Asp + Ile209Ala  
Gly135Asp + Gly139Pro + Pro169Asp + Trp208Glu  
Asn 66Glu + Thr167Gly + Trp208Glu + Pro214Asp  
Asn 66Asp + Ser109Asp + Trp208Gly + Pro214Glu  
Gly 69Glu + Asn140Ser + Ser192Glu + Pro214Asp  
Leu104Gly + Gly139Glu + Ser191Asp + Thr224Asp  
Asn106Gln + Thr113Asp + Asn163Asp + Asn166Glu  
Leu134Glu + Ser207Asp + Trp208Pro + Tyr210Glu  
Ser191Glu + Thr195Asn + Tyr218Pro + Leu221Glu  
Asn170Asp + Phe193Ser + Ser220Glu + Thr224Asp  
Asp105Glu + Ala114Glu + Ser192Glu + Thr224Asn  
Gly 67Asp + Gly108Asp + Ser207Asp + Tyr210Cys  
Asn 68Asp + Gly110Glu + Thr113Gly + Leu221Ala  
Leu104Ile + Ala114Glu + Ser220Glu + Gly223Glu  
Ser107Asp + Ser220Glu + Leu221Pro + Gly223Asp  
Val103Asp + Val138Glu + Ser141Glu + Gly206Gln  
Val138Glu + Ser141Asp + Tyr210Gln + Ser220Asp  
Asn 68Asp + Val115Gly + Thr217Asp + Ser220Glu  
Asn 66Asp + Leu104Gly + Ala168Thr + Ser216Glu  
Val103Glu + Asn106Glu + Thr113Pro + Ser220Asp  
Asp105Glu + Thr113Asp + Gly139Asn + Ser141Glu  
Ser 70Glu + Asp105Glu + Gly139Asp + Tyr210Ala  
Asn 66Glu + Tyr210Asp + Thr217Glu + Leu221Ala  
Gly108Glu + Gly135Ser + Tyr210Asp + Thr217Glu  
Gly 69Asp + Gly108Gln + Trp112Asp + Ser141Glu  
Ser107Glu + Gly110Pro + Thr217Asp + Ser220Asp  
Gly135Glu + Thr217Glu + Tyr218Val + Ser220Glu  
Asn163Asp + Tyr171Thr + Thr217Asp + Ser220Asp  
Ala114Thr + Thr137Glu + Thr217Asp + Ser220Asp  
Asn166Glu + Thr212Pro + Thr217Asp + Ser220Asp  
Gly 67Asp + Ser107Asp + Tyr210Asn + Ser222Glu  
Asn140Ser + Trp208Gly + Thr217Glu + Leu221Asp  
Asn166Gln + Thr215Pro + Thr217Glu + Leu221Asp  
Val115Gln + Ala168Gln + Phe193Asp + Leu221Asp  
Trp208Ile + Pro214Ser + Ser220Glu + Thr224Glu  
Gly108Glu + Thr137Glu + Asn163Asp + Trp208Ser  
Gly139Pro + Ala164Glu + Ser191Asp + Pro214Glu

Thr 73Asp + Thr111Pro + Asn166Asp + Ser191Asp  
 Ser 70Glu + Ala168His + Thr195Glu + Ser216Asp  
 Ser109Glu + Thr137Asp + Gly139Ser + Ala164Gln  
 Asn170Gln + Tyr210Asp + Pro214Glu + Gly223Ser  
 Ser 70Glu + Gly139Asn + Ala164Glu + Ser220Asp  
 Asn 66Asp + Thr113Glu + Ser141Asp + Trp208Gly  
 Asn 66Asp + Gly108Glu + Thr215Gly + Leu221Ser  
 Asn 68Ser + Thr 73Glu + Asn166Glu + Leu221Asp  
 Thr 73Asp + Ser192Asp + Ser220Asp + Leu221Met  
 Ser107Asp + Thr111Asp + Ser192Glu + Thr217Ser

TABLE 35

## Multi-loop Quintuple Mutation Variants

Asn 66Gln + Gly 67Ser + Leu104Gly + Tyr210Pro +  
 Thr217Glu  
 Gly 69Ser + Val103Gln + Trp112Cys + Thr217Gly +  
 Gly223Asp  
 Val115Pro + Leu134Ile + Ala164Glu + Phe193Asn +  
 Trp208Gln  
 Leu104Gly + Thr113Ser + Ala168Glu + Asn170Ser +  
 Ala219Gly  
 Gly 69Ser + Ser109Asp + Thr111Gln + Val115Ala +  
 Tyr210Pro  
 Val103Thr + Gly108Glu + Val115Pro + Tyr171Ser +  
 Trp208Asn  
 Leu134Ala + Val138Cys + Asn163Gln + Tyr171Ser +  
 Ser220Asp  
 Asn 66Ser + Val115Gly + Ser191Glu + Trp208Ile +  
 Thr217Ser  
 Gly 72Asn + Trp112Phe + Val115Ala + Asn166Gln +  
 Trp208Gly  
 Leu134His + Gly165Gln + Trp208Pro + Tyr210Val +  
 Thr217Glu  
 Leu104Pro + Tyr171Leu + Trp208Leu + Ala219Glu +  
 Leu221Ser  
 Asn166Glu + Thr167Pro + Tyr210Pro + Tyr213Ile +  
 Pro214Asn  
 Gly139Gln + Asn170Glu + Trp208Cys + Thr215Ser +  
 Leu221Asn  
 Ala114Glu + Gly135Ser + Ala168Thr + Ala203Thr +  
 Trp208Met  
 Ala164His + Ala168His + Phe193Ala + Thr217Ser +  
 Ser220Asp  
 Gly 67Pro + Gly 69Ser + Ser191Asp + Tyr218Val +  
 Thr224Ser  
 Gly139Asn + Ala164Pro + Phe193Pro + Thr217Asn +  
 Leu221Cys  
 Leu104Met + Ser207Asp + Trp208Ala + Thr212Gly +  
 Thr217Pro  
 Leu134Val + Trp208Thr + Pro214Gly + Leu221Ile +  
 Thr224Asp



Ser109Asp + Ala114Asn + Val115Asn + Gly165Gln +  
Tyr210Ser  
Leu104Ser + Gly110Pro + Gly136Glu + Ala168Thr +  
Ala219Gln  
Gly110Pro + Leu134Pro + Asn166Asp + Asn170Ser +  
Trp208Asn  
Thr113Gln + Pro169Gly + Asn170Gln + Tyr218Gln +  
Leu221Ser  
Thr137Ser + Thr195Gln + Ser207Glu + Trp208Gln +  
Thr217Pro  
Gly 69Gln + Val103Gly + Trp112Met + Trp208Asn +  
Pro214Gly  
Gly136Pro + Gly162Ser + Trp208Ile + Tyr210Gly +  
Pro214Gln  
Thr137Asp + Gly165Gln + Thr195Ser + Trp208Pro +  
Thr212Ser  
Asn 66Ser + Pro169Ser + Tyr210Asn + Tyr218Asn +  
Gly223Glu  
Ser 70Glu + Asn106Gln + Val138Ala + Ala168Gln +  
Leu221Val  
Gly 69Ser + Thr113Asp + Ala114Gln + Trp208Thr +  
Ala219Ser  
Thr113Gln + Ala168Asp + Pro169Ser + Asn170Ser +  
Ala219Thr  
Gly139Ser + Asn170Gln + Tyr171Leu + Trp208Cys +  
Thr215Ser  
Gly 67Ser + Ala114Asn + Gly139Ser + Tyr210Thr +  
Tyr218Ser  
Trp112Glu + Ala164Pro + Tyr171Pro + Thr195Asn +  
Thr217Ser  
Gly 67Glu + Val115Asn + Gly162Gln + Gly165Gln +  
Phe193Val  
Gly135Ser + Tyr171Cys + Trp208His + Tyr210Met +  
Leu221Val  
Gly 69Asn + Asn106Ser + Asn166Glu + Tyr210Cys +  
Leu221Gly  
Gly135Ser + Tyr171Asn + Trp208His + Ser216Glu +  
Leu221Gly  
Gly 69Asn + Ser109Asp + Gly165Asn + Phe193Asn +  
Trp208His  
Pro169Ser + Ser207Glu + Pro214Asn + Thr215Asn +  
Thr217Gly  
Ser 70Asp + Asn163Ser + Tyr210Val + Tyr213Asn +  
Leu221Ala  
Gly108Gln + Gly110Ser + Gly135Gln + Thr137Asn +  
Trp208Glu  
Asn 68Ser + Ala164Gln + Asn170Glu + Thr217Ser +  
Gly223Ser  
Gly 69Ser + Trp112Cys + Ala164Gly + Leu221Ser +  
Gly223Glu  
Gly 69Glu + Gly 72Gln + Thr137Pro + Trp208Ile +  
Tyr218Pro  
Val103Gln + Val138His + Gly162Ser + Asn166Asp +

Trp208His  
 Val115Gln + Leu134Cys + Ala168Ser + Pro169Asp +  
 Tyr210Ser  
 Gly 67Gln + Trp112Leu + Phe193His + Thr195Glu +  
 Thr217Ser  
 Gly 67Gln + Leu104Asn + Ala164Glu + Ala204Ser +  
 Pro205Ser  
 Gly135Ser + Val138Ser + Asn140Glu + Tyr210Leu +  
 Thr217Gly  
 Gly 67Asn + Thr 73Ser + Tyr171Leu + Phe193Tyr +  
 Tyr210Pro  
 Gly135Pro + Trp208His + Tyr210Leu + Ala219Ser +  
 Leu221Gln  
 Asn 68Gln + Asn106Glu + Thr137Gln + Pro169Gln +  
 Thr215Gln  
 Thr137Asp + Val138Gln + Trp208Ile + Tyr218Leu +  
 Leu221Cys  
 Gly 67Asn + Gly165Asn + Phe193Tyr + Pro205Ser +  
 Trp208Cys  
 Asn 66Ser + Thr 73Ser + Ser107Asp + Ala168Ser +  
 Tyr210Cys  
 Thr137Gly + Tyr210Pro + Thr215Pro + Ser216Asp +  
 Thr217Ser  
 Thr 73Pro + Trp112Asp + Gly139Pro + Thr215Pro +  
 Leu221His  
 Leu104Cys + Thr111Pro + Trp208Pro + Leu221Cys +  
 Ser222Asp  
 Thr113Gln + Ala204His + Trp208Phe + Thr217Asp +  
 Leu221Cys  
 Thr111Gly + Gly139Gln + Tyr171Leu + Tyr210Met +  
 Thr217Glu  
 Gly110Asn + Val115Gln + Pro169Gln + Thr195Gly +  
 Ser220Asp  
 Tyr171Asp + Gly206Ser + Thr217Gln + Ala219Gly +  
 Leu221Thr  
 Ser109Glu + Thr111Ser + Val115Cys + Val138Cys +  
 Tyr171Ser  
 Leu104His + Asn163Asp + Trp208Thr + Tyr210Cys +  
 Thr217Asn  
 Gly136Asn + Gly139Pro + Ser141Glu + Pro169Gln +  
 Ala219His  
 Gly 69Ser + Gly136Ser + Thr137Asn + Thr195Pro +  
 Ala219Glu  
 Asn106Asp + Gly162Ser + Asn163Gln + Pro169Gly +  
 Leu221Pro  
 Ala168Gly + Phe193Met + Pro205Asn + Tyr210Asn +  
 Leu221Asn  
 Gly 69Pro + Gly139Asp + Ala164Gly + Tyr213Ser +  
 Thr217Ser  
 Gly 72Ser + Leu104Gln + Asn166Glu + Pro169Asn +  
 Thr215Gly  
 Leu134Cys + Trp208Asn + Tyr210Ala + Tyr218Gly +  
 Thr224Gly

Leu104Gln + Thr195Asn + Trp208Val + Tyr210Pro +  
Leu221Cys  
Gly 67Pro + Thr 73Asn + Trp208Pro + Ala219Asn +  
Ser220Asp  
Thr 73Gln + Gly108Pro + Gly139Pro + Ser192Glu +  
Gly223Gln  
Asn 68Glu + Thr 73Gln + Pro169Gln + Tyr210Pro +  
Leu221Met  
Leu104His + Thr113Gln + Asn140Gln + Thr167Asn +  
Phe193Asp  
Gly 67Pro + Asn 68Ser + Gly139Asp + Thr215Gly +  
Thr217Pro  
Gly136Ser + Val138Met + Thr167Gln + Tyr218Asn +  
Ser220Asp  
Asp105Glu + Gly110Ser + Trp112His + Tyr210Gln +  
Ala219Thr  
Asn 68Asp + Asn106Gln + Ala164Ser + Thr195Gln +  
Trp208Cys  
Leu104Val + Leu134Ala + Thr137Asn + Asn170Asp +  
Leu221Ile  
Gly 67Ser + Leu134Met + Trp208Ala + Tyr210Ser +  
Thr217Pro  
Ala114Gly + Gly135Asp + Gly139Ser + Asn170Ser +  
Thr217Ser  
Thr111Asn + Phe193Ser + Pro205Gln + Trp208Thr +  
Leu221Glu  
Ser109Asp + Val138Asn + Phe193Ala + Trp208Cys +  
Thr212Gln  
Ser 70Asp + Asn166Ser + Ile209Pro + Tyr210Met +  
Leu221Ser  
Asn106Ser + Leu134Cys + Gly136Ser + Ala168Thr +  
Tyr210Glu  
Gly 67Gln + Leu134Gln + Gly135Asp + Phe193His +  
Thr224Gly  
Asn 68Ser + Leu104Ala + Gly162Asp + Tyr171Met +  
Thr217Gly  
Asn163Glu + Thr212Gln + Thr215Asn + Thr217Asn +  
Leu221Met  
Gly 69Gln + Asn106Gln + Ser207Glu + Trp208Thr +  
Thr217Gly  
Gly139Glu + Ala168His + Thr195Gly + Leu221His +  
Thr224Asn  
Gly 72Ser + Ala114Asn + Leu134His + Pro169Glu +  
Trp208Tyr  
Val138Ser + Ala164Asn + Tyr210Cys + Tyr218Leu +  
Ser220Asp  
Leu104Gln + Tyr171Glu + Trp208Gly + Thr217Gln +  
Ala219His  
Asn106Ser + Tyr171Gly + Phe193Gln + Thr215Gln +  
Ala219Gln  
Gly 72Ser + Gly108Asn + Trp112Ile + Asn166Glu +  
Leu221Cys  
Asn 68Ser + Leu104Thr + Thr215Ser + Ala219Thr +

Thr224Asp  
Val138Ser + Asn163Asp + Phe193Cys + Pro205Asn +  
Thr217Ser  
Gly 67Ser + Tyr210Pro + Tyr218Thr + Ser220Asp +  
Leu221Ser  
Gly135Asn + Gly136Gln + Gly162Ser + Tyr210Asn +  
Pro214Asn  
Thr195Ser + Trp208Pro + Tyr210Gly + Ala219Ser +  
Leu221Ile  
Asn 68Glu + Thr111Pro + Asn163Ser + Phe193Tyr +  
Leu221Val  
Gly 67Ser + Thr 73Pro + Asn170Glu + Tyr210Leu +  
Leu221Val  
Gly135Asn + Gly139Ser + Phe193Asp + Thr217Gly +  
Leu221Gly  
Gly136Gln + Gly139Gln + Gly165Ser + Tyr210Asp +  
Thr217Pro  
Ser141Glu + Gly162Pro + Thr167Gly + Tyr210Ile +  
Thr217Ser  
Leu104Pro + Gly110Pro + Gly136Pro + Pro205Asn +  
Thr217Glu  
Val115Ala + Ala164Thr + Ser192Asp + Trp208Leu +  
Tyr210Met  
Thr 73Asn + Val103Asn + Thr215Asn + Thr217Gln +  
Ser222Asp  
Val103Pro + Trp208Tyr + Ile209Cys + Tyr210Thr +  
Thr217Gln  
Trp112Phe + Ala114Ser + Pro169Gln + Ser220Glu +  
Leu221His  
Gly108Asn + Leu134Met + Tyr210Ser + Tyr213Ile +  
Pro214Glu  
Ala168Pro + Trp208His + Tyr210Ala + Ser220Glu +  
Leu221Gln  
Gly139Pro + Asn140Glu + Ser141Asp + Asn166Ser +  
Trp208Thr  
Val103Asn + Thr111Gly + Tyr210Gln + Ser216Asp +  
Thr217Glu  
Val138His + Gly139Asn + Ser207Asp + Trp208Glu +  
Leu221Gln  
Ala168Asn + Pro169Gly + Thr217Pro + Ser220Asp +  
Leu221Asp  
Tyr210Leu + Ala219Gly + Ser220Asp + Leu221Asp +  
Gly223Ser  
Val103Ala + Pro169Asn + Thr217Gln + Ser220Asp +  
Leu221Asp  
Thr 73Asn + Asn170Asp + Tyr171Glu + Tyr210Ile +  
Leu221Ser  
Gly136Ser + Ser191Asp + Ser192Glu + Tyr218Cys +  
Leu221Asn  
Thr 73Pro + Val115Ala + Ser192Asp + Phe193Glu +  
Trp208Gln  
Thr137Gln + Gly162Asp + Asn163Asp + Ala203His +  
Pro205Gln

Asn163Gln + Tyr210Asp + Thr215Pro + Ser220Glu +  
Gly223Pro  
Asp105Glu + Asn106Glu + Ser107Asp + Trp208Phe +  
Tyr210Ser  
Trp112Gly + Phe193Gln + Ser207Asp + Trp208His +  
Ser222Asp  
Gly108Asn + Ser207Glu + Tyr213Thr + Thr215Pro +  
Ser222Asp  
Leu134Met + Ser207Asp + Trp208Val + Tyr210His +  
Ser222Asp  
Gly 69Glu + Asn106Asp + Ala114His + Ala168Ser +  
Thr217Gln  
Leu104Pro + Trp208Glu + Thr212Asn + Thr215Ser +  
Ser222Asp  
Thr111Pro + Thr137Asn + Pro169Gln + Tyr210Glu +  
Ala219Glu  
Thr 73Gly + Val115Gly + Trp208Glu + Tyr210Asp +  
Ser220Glu  
Gly135Gln + Gly136Glu + Asn170Ser + Tyr171Glu +  
Trp208Gln  
Thr 73Ser + Ser107Asp + Ser109Asp + Gly136Pro +  
Tyr210Gly  
Leu134Pro + Pro169Gln + Pro214Glu + Ser216Glu +  
Thr217Gly  
Asp105Glu + Ser109Glu + Pro169Gly + Tyr171Gln +  
Thr212Ser  
Gly135Pro + Val138Ala + Ser207Asp + Tyr210Thr +  
Gly223Glu  
Gly135Pro + Ser207Glu + Trp208Ala + Tyr210Pro +  
Gly223Glu  
Pro169Gln + Phe193Val + Tyr210Glu + Thr215Asn +  
Leu221Asp  
Ala114Gln + Gly136Ser + Ala219Asn + Ser220Asp +  
Ser222Asp  
Leu134Ser + Thr167Gly + Ser220Asp + Leu221Pro +  
Ser222Asp  
Gly108Gln + Trp112Leu + Tyr218Glu + Ser220Asp +  
Leu221Glu  
Gly108Pro + Ala114Asn + Ser207Asp + Tyr210Glu +  
Ser220Glu  
Thr137Glu + Gly162Glu + Pro169Asn + Asn170Ser +  
Tyr171Asp  
Asn 68Gln + Gly162Glu + Ala164Thr + Gly165Asp +  
Tyr171Ala  
Thr137Pro + Gly139Asp + Asn170Glu + Trp208Met +  
Tyr210Thr  
Asn 68Gln + Val138Ser + Gly162Pro + Ala164Glu +  
Asn170Asp  
Asn 68Gln + Ser207Glu + Trp208Thr + Thr217Ser +  
Ser220Asp  
Val138Ala + Phe193Thr + Ser207Asp + Tyr210Met +  
Ser220Asp  
Phe193Ala + Trp208Glu + Ile209Ser + Leu221Cys +

Gly223Glu  
 Thr111Gly + Phe193Gln + Ser207Asp + Ser220Asp +  
 Gly223Glu  
 Ser 70Asp + Asp105Glu + Gly108Glu + Thr111Ser +  
 Tyr210Val  
 Gly136Glu + Gly162Asp + Gly165Ser + Ala168Pro +  
 Thr195Glu  
 Leu104Gly + Gly165Glu + Pro169Glu + Thr217Asn +  
 Leu221Ala  
 Thr195Asn + Tyr210Asp + Thr217Glu + Ser220Glu +  
 Gly223Pro  
 Trp208Phe + Tyr210Asp + Tyr213Cys + Thr217Glu +  
 Ser220Glu  
 Ser 70Asp + Gly108Glu + Tyr171Gly + Thr217Gln +  
 Leu221Pro  
 Thr195Gly + Ser207Glu + Tyr210Glu + Tyr213Cys +  
 Ala219Ser  
 Asn 68Glu + Asp105Glu + Gly110Asp + Thr111Pro +  
 Asn140Ser  
 Ser 70Asp + Val103Gln + Pro214Gln + Ser220Asp +  
 Leu221Glu  
 Asn 66Asp + Thr 73Glu + Asn106Ser + Trp208His +  
 Leu221Thr  
 Ala164Asp + Thr167Asp + Tyr171Pro + Ala219Gln +  
 Leu221Thr  
 Ala114His + Ala219Gly + Ser220Glu + Ser222Glu +  
 Thr224Glu  
 Asn106Asp + Ser109Asp + Thr111Asp + Thr137Ser +  
 Pro205Asn  
 Leu104Cys + Asn163Glu + Ser192Asp + Ala219Pro +  
 Ser222Glu  
 Gly162Asp + Ala164Gln + Ser207Asp + Trp208Tyr +  
 Gly223Asp  
 Ser 70Asp + Ser107Glu + Ser109Glu + Val115Cys +  
 Gly135Ser  
 Ala114Gly + Ala219Asp + Ser220Glu + Leu221Asn +  
 Gly223Glu  
 Asn 66Asp + Asp105Glu + Val115Gln + Thr137Ser +  
 Trp208His  
 Gly 69Glu + Thr 73Glu + Val103His + Ser107Asp +  
 Leu221Ala  
 Gly108Pro + Trp112Gln + Gly162Asn + Ser220Glu +  
 Gly223Asp  
 Asn 68Asp + Asn170Gln + Tyr171Pro + Thr215Asp +  
 Ser216Asp  
 Ser 70Asp + Tyr171Ser + Thr195Gly + Tyr210Asp +  
 Ser220Glu  
 Thr113Gly + Gly162Ser + Trp208Glu + Ser220Asp +  
 Thr224Asp  
 Ser 70Glu + Asn140Ser + Ala203Gln + Ala219Glu +  
 Gly223Pro  
 Trp112Pro + Asn170Ser + Ser216Asp + Thr217Asp +  
 Ser220Asp

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Thr 73Asn + Val138Asp + Pro169Glu + Asn170Ser +  
Thr217Gly  
Ser 70Asp + Asp105Glu + Gly162Pro + Trp208Val +  
Thr217Ser  
Gly 67Asn + Asn140Ser + Ala219Glu + Leu221Met +  
Ser222Glu  
Gly 72Ser + Gly110Pro + Trp112Ala + Tyr210Asp +  
Thr217Glu  
Gly108Pro + Trp112Leu + Leu134Cys + Gly135Asp +  
Ala164Asp  
Asn 66Gln + Trp208Ala + Thr217Glu + Ser220Glu +  
Leu221Thr  
Thr 73Gly + Leu134Ser + Thr217Glu + Ser220Glu +  
Leu221Asn  
Gly 67Ser + Ala168Gly + Pro214Gln + Thr217Glu +  
Ser220Asp  
Val115Cys + Gly139Asn + Tyr210Cys + Thr217Asp +  
Ser220Asp  
Leu134Glu + Thr137Glu + Thr195Asn + Trp208Ser +  
Leu221Ser  
Ala164Gln + Tyr210Val + Thr215Asp + Thr217Glu +  
Ser220Asp  
Gly 67Asp + Ser 70Asp + Thr217Gly + Tyr218Asp +  
Leu221Ile  
Ser 70Asp + Thr111Asn + Trp208Cys + Thr217Glu +  
Ser220Glu  
Leu134Glu + Gly162Ser + Asn166Gln + Ser207Asp +  
Gly223Asp  
Gly162Pro + Thr167Asn + Pro169Glu + Ser191Glu +  
Phe193Asp  
Ser 70Glu + Trp112Tyr + Ser207Glu + Tyr218Cys +  
Leu221Glu  
Asn 66Glu + Leu104Glu + Ser107Asp + Trp208Ser +  
Thr215Pro  
Leu104Ala + Gly135Asp + Thr195Asp + Tyr213Ile +  
Thr217Gly  
Thr167Gln + Ser192Glu + Ser207Glu + Trp208Cys +  
Ser220Asp  
Gly165Pro + Ser191Asp + Trp208Gly + Ser220Asp +  
Leu221Glu  
Gly135Glu + Asn163Asp + Tyr213Met + Leu221His +  
Ser222Asp  
Thr111Asp + Gly136Glu + Gly162Glu + Ala164Asn +  
Pro214Ser  
Thr113Gln + Tyr213Thr + Ser216Glu + Thr217Glu +  
Leu221Glu  
Ser 70Glu + Gly108Glu + Ala164Gly + Gly165Gln +  
Leu221Asp  
Ser 70Glu + Leu104Gln + Ser220Asp + Leu221His +  
Gly223Pro  
Gly165Asp + Pro169Ser + Ser207Glu + Trp208Gly +  
Ser222Asp  
Ala168Pro + Pro169Asn + Thr215Asp + Thr217Asp +

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Leu221Glu  
Gly110Pro + Thr113Ser + Gly165Glu + Trp208Asp +  
Ser222Asp  
Gly110Glu + Thr113Asp + Gly139Glu + Trp208Tyr +  
Tyr210Ala  
Asn 68Glu + Asn106Glu + Tyr171His + Tyr210Gln +  
Ser216Glu  
Ala164Glu + Ala168His + Ala219Glu + Ser220Asp +  
Leu221Cys  
Asn 68Asp + Tyr210Gln + Ala219Glu + Ser220Glu +  
Leu221Ser  
Thr 73Asp + Asn163Ser + Ala219His + Ser222Glu +  
Gly223Glu  
Gly 67Asp + Asn 68Glu + Ala168Gln + Pro169Glu +  
Phe193Asn  
Gly110Asp + Gly139Gln + Asn140Glu + Ser141Glu +  
Tyr218Asn  
Ser109Glu + Ser216Glu + Thr217Asp + Tyr218Asn +  
Leu221His  
Gly135Pro + Gly136Ser + Phe193Asp + Ser216Asp +  
Thr217Glu  
Gly165Gln + Ser192Glu + Ala203Ser + Ser216Asp +  
Thr217Asp  
Gly 67Gln + Leu134His + Gly136Asp + Ser207Asp +  
Trp208Glu  
Val103Ser + Ser207Asp + Trp208Glu + Thr217Glu +  
Leu221Cys  
Gly 69Pro + Asp105Glu + Asn106Asp + Asn166Asp +  
Ile209Thr  
Trp112Tyr + Asn170Glu + Trp208Gln + Ser220Glu +  
Leu221Glu  
Thr137Gly + Gly139Gln + Gly162Asp + Ser220Glu +  
Leu221Asp  
Thr113Asp + Ala164Gly + Ser191Glu + Ser192Glu +  
Thr195Gly  
Ser107Glu + Thr113Glu + Ala114Asp + Tyr210Pro +  
Leu221Ala  
Asn 66Asp + Gly 67Asp + Leu134Pro + Tyr210Asn +  
Ser222Asp  
Asn 68Gln + Leu104Ala + Pro169Asp + Asn170Glu +  
Leu221Asp  
Asn106Glu + Ser107Asp + Gly139Asp + Ala203Asn +  
Thr217Ser  
Thr 73Gly + Thr137Glu + Tyr210Asp + Thr217Gln +  
Ser220Glu  
Trp112Ile + Gly136Glu + Phe193Thr + Tyr210Glu +  
Ser220Glu  
Trp112Ala + Asn163Ser + Ser192Glu + Tyr210Glu +  
Ser220Glu  
Ser 70Glu + Thr 73Ser + Val103Asp + Pro214Glu +  
Tyr218Gly  
Asn106Glu + Ser109Glu + Thr195Gln + Trp208Pro +  
Pro214Glu



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Asn 68Gln + Val103Asp + Thr137Gln + Ser207Asp +  
     Ser222Asp  
 Thr137Asp + Ser207Glu + Thr217Gly + Leu221Pro +  
     Ser222Asp  
 Gly 69Asp + Thr 73Glu + Thr111Pro + Gly139Asn +  
     Ser220Asp  
 Asn 66Glu + Ser 70Asp + Pro214Gly + Ser220Glu +  
     Leu221Ser  
 Ser 70Glu + Leu104Cys + Tyr210Ser + Thr215Glu +  
     Ser220Glu  
 Thr167Pro + Ser216Glu + Ala219Glu + Leu221Met +  
     Ser222Asp  
 Val138Asp + Asn166Glu + Thr167Asp + Ala168Thr +  
     Leu221Ala  
 Leu134Pro + Ser141Glu + Trp208Glu + Thr217Ser +  
     Ser220Glu  
 Gly135Glu + Tyr171Asn + Pro205Gln + Trp208Glu +  
     Ser220Asp  
 Trp112Asn + Thr167Glu + Ala168Gly + Trp208Asp +  
     Ser220Glu  
 Asn140Asp + Pro169Gln + Trp208Glu + Thr215Gln +  
     Ser220Glu  
 Asn106Gln + Gly136Asp + Trp208Glu + Thr217Ser +  
     Ser220Glu  
 Asn106Asp + Thr111Asn + Thr167Gly + Tyr210Asp +  
     Ala219Asp  
 Asn 66Gln + Pro169Asp + Tyr171Glu + Pro214Asn +  
     Gly223Glu  
 Trp112Gly + Asn163Asp + Thr167Glu + Ser191Glu +  
     Gly223Ser  
 Trp112Asp + Thr113Ser + Ala114Glu + Thr217Glu +  
     Gly223Gln  
 Asn 66Asp + Thr 73Gly + Val103Asp + Thr111Pro +  
     Thr195Asn  
 Asn 68Asp + Trp112Met + Trp208Asn + Pro214Asp +  
     Ser220Asp  
 Asp105Glu + Ser109Glu + Asn163Glu + Asn166Ser +  
     Tyr218Gly  
 Gly110Gln + Trp112Asp + Leu134Pro + Pro214Asp +  
     Thr217Asp  
 Gly 67Pro + Thr113Glu + Gly135Asp + Gly162Asp +  
     Thr215Gln  
 Ser 70Glu + Thr195Ser + Pro214Ser + Ser220Glu +  
     Gly223Glu  
 Gly110Pro + Val138Glu + Trp208Glu + Tyr210Glu +  
     Thr217Gly  
 Gly108Asn + Asn163Glu + Ala219Asp + Leu221Val +  
     Thr224Asp  
 Thr 73Asp + Gly108Asn + Ser109Glu + Pro214Glu +  
     Thr217Asn  
 Thr137Asp + Gly139Glu + Asn170Ser + Trp208Cys +  
     Tyr210Asp  
 Trp112Phe + Gly139Glu + Tyr213Thr + Ala219Glu +

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Leu221Glu  
Leu104Pro + Pro169Glu + Tyr210Glu + Leu221Asp +  
Thr224Gly  
Asp105Glu + Gly165Glu + Thr167Gln + Ala168Glu +  
Tyr210Pro  
Thr137Asp + Trp208Asn + Tyr218Ser + Ser220Asp +  
Ser222Asp  
Gly 69Glu + Thr113Gln + Thr217Ser + Ser220Glu +  
Ser222Glu  
Leu104His + Thr113Asp + Ser207Asp + Thr217Ser +  
Leu221Glu  
Ala114Thr + Gly135Asp + Ser207Asp + Thr217Ser +  
Leu221Asp  
Gly 69Ser + Leu104Asp + Asn106Glu + Ser191Glu +  
Thr217Gly  
Val103Ala + Tyr171Val + Ser207Asp + Ser216Asp +  
Tyr218Asp  
Gly 69Ser + Leu134Ile + Gly162Asp + Ser207Glu +  
Ser220Glu  
Gly 72Pro + Thr113Glu + Asn166Asp + Thr167Asp +  
Thr217Gln  
Asn166Glu + Thr167Asp + Ser207Glu + Thr217Gly +  
Leu221Val  
Thr111Asn + Trp112Glu + Val138Gly + Asn163Asp +  
Tyr171Glu  
Gly 67Pro + Asn106Glu + Leu134Pro + Thr137Asp +  
Pro169Glu  
Thr195Glu + Ser207Asp + Ser220Asp + Leu221Val +  
Thr224Gly  
Asn 68Asp + Gly 69Gln + Gly162Asp + Pro169Ser +  
Gly223Asp  
Thr 73Ser + Gly165Asn + Ser207Asp + Tyr210Asp +  
Ser216Glu  
Val103Glu + Ala164Asp + Asn170Glu + Trp208Ala +  
Tyr210Ile  
Ala114Thr + Ala164Asp + Asn170Glu + Tyr210Ala +  
Thr215Asp  
Asn 68Glu + Gly139Pro + Ala164Pro + Ser191Asp +  
Pro214Glu  
Asn 68Glu + Ala164Glu + Gly165Ser + Tyr210Gly +  
Pro214Asp  
Gly 67Ser + Thr167Gly + Ser207Glu + Ser216Asp +  
Ser220Asp  
Asp105Glu + Gly110Asn + Ser207Asp + Ala219Gly +  
Ser220Glu  
Val103Gly + Asn140Asp + Ser207Asp + Tyr213Asn +  
Ser220Asp  
Ser109Glu + Gly165Asn + Ser207Glu + Tyr210Cys +  
Ser220Asp  
Gly110Asn + Asn140Glu + Ser207Glu + Thr217Ser +  
Ser220Glu  
Gly110Asp + Gly136Pro + Gly206Gln + Ser207Glu +  
Ser220Glu

Thr113Gln + Leu134Ile + Ser192Glu + Thr195Asp +  
Ser220Asp  
Gly136Glu + Trp208Glu + Thr217Gly + Leu221Asn +  
Gly223Asp  
Ser107Asp + Gly110Asp + Leu134Ser + Asn140Glu +  
Tyr210Thr  
Thr167Glu + Trp208Asp + Thr217Asn + Ala219Asp +  
Gly223Asn  
Gly 69Glu + Ala114Asp + Thr195Ser + Trp208Ser +  
Pro214Glu  
Val103Asp + Thr113Glu + Phe193Tyr + Trp208Asp +  
Ala219Pro  
Asn106Asp + Gly136Ser + Val138Met + Ser191Asp +  
Ser222Asp  
Gly135Asp + Gly139Glu + Tyr210Ala + Thr217Ser +  
Ser222Asp  
Thr113Asp + Asn140Ser + Ala203Gln + Ser207Glu +  
Tyr210Glu  
Gly108Glu + Thr113Gln + Ser207Glu + Tyr210Asp +  
Pro214Asn  
Gly135Glu + Asn163Asp + Tyr210Met + Tyr218Pro +  
Ser220Asp  
Asn163Asp + Ala164Thr + Ser191Glu + Thr217Asp +  
Thr224Gln  
Asn 68Glu + Leu104Asp + Val115Thr + Ala164Asp +  
Pro214Gln  
Thr 73Gly + Ser107Glu + Gly108Pro + Leu221Glu +  
Thr224Asp  
Gly 67Ser + Gly162Asp + Thr167Asp + Ser207Glu +  
Thr217Ser  
Gly 67Gln + Ser109Glu + Val138Ser + Pro214Glu +  
Ala219Glu  
Ser107Glu + Gly135Glu + Trp208His + Thr217Ser +  
Thr224Glu  
Asn 66Glu + Asp105Glu + Val138His + Ser192Glu +  
Tyr210Ile  
Asn 66Glu + Asp105Glu + Ala219Gln + Ser220Glu +  
Leu221Asn  
Gly 69Ser + Ser 70Glu + Gly110Glu + Thr217Asp +  
Leu221Ala  
Tyr171Glu + Ser192Asp + Ser207Glu + Trp208Pro +  
Tyr210Met  
Gly 67Gln + Asp105Glu + Ala164Asp + Pro169Asn +  
Gly223Asp  
Thr 73Asp + Ser141Asp + Trp208Leu + Ser216Glu +  
Thr217Pro  
Asn 66Asp + Trp208Gln + Ile209Thr + Tyr210Val +  
Ser216Glu  
Asn 66Ser + Val103Glu + Asn106Asp + Ser191Glu +  
Tyr210Ala  
Asn 68Gln + Leu134Glu + Ser192Glu + Leu221Gly +  
Ser222Glu  
Ser 70Asp + Asp105Glu + Gly108Ser + Gly139Glu +

Thr217Gly  
Gly 67Ser + Asn106Asp + Gly165Gln + Ala219Asp +  
Ser222Glu  
Asn106Gln + Leu134Met + Asn140Asp + Ala219Asp +  
Ser222Asp  
Gly 69Glu + Val103Gln + Leu134Asp + Thr167Gln +  
Trp208Gly  
Thr137Glu + Gly165Glu + Tyr210Ser + Thr217Ser +  
Leu221Pro  
Ser107Asp + Gly162Asp + Thr167Asp + Tyr171Cys +  
Trp208Ile  
Asn106Glu + Trp112Asp + Leu134Gly + Ser141Glu +  
Phe193Ser  
Ala114Thr + Asn140Asp + Phe193Thr + Thr217Glu +  
Ser220Glu  
Thr113Ser + Val115Asp + Leu134Val + Thr217Asp +  
Ser220Glu  
Gly110Asp + Asn170Gln + Thr217Glu + Tyr218Ala +  
Ser220Asp  
Thr167Asp + Phe193Thr + Thr217Asp + Ala219Gln +  
Ser220Asp  
Gly108Glu + Pro214Asn + Thr217Glu + Ser220Asp +  
Leu221Val  
Asn106Gln + Gly135Glu + Val138His + Thr217Glu +  
Ser220Glu  
Gly110Glu + Tyr210Met + Thr217Glu + Ser220Asp +  
Leu221His  
Gly 67Glu + Ser107Glu + Gly110Ser + Thr113Glu +  
Thr215Ser  
Gly 67Glu + Ser107Glu + Thr111Pro + Ala168His +  
Ser220Glu  
Thr111Asp + Gly136Asp + Trp208Glu + Tyr210Met +  
Thr217Gln  
Asp105Glu + Gly162Ser + Asn166Asp + Ser191Asp +  
Trp208Phe  
Asp105Glu + Asn166Glu + Ser191Glu + Trp208Ala +  
Leu221His  
Gly 67Glu + Val103Glu + Ala164Asn + Ser207Asp +  
Tyr218Ala  
Gly 72Asn + Leu104Asp + Asn106Gln + Thr113Asp +  
Thr217Asp  
Ser 70Asp + Gly135Glu + Ala168Glu + Tyr210Ile +  
Ala219Gly  
Asn106Gln + Ser109Glu + Thr113Asp + Thr137Glu +  
Trp208Asn  
Ala114Ser + Val138Thr + Pro169Asn + Phe193Asp +  
Trp208Glu  
Asn 68Asp + Val138Asp + Ala164Gly + Ala168Glu +  
Thr217Gly  
Gly 69Asn + Val138Asp + Ala168Glu + Ser207Asp +  
Thr217Pro  
Gly110Ser + Thr111Gln + Ala164Glu + Thr215Ser +  
Ser222Asp

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Leu104Ser + Gly108Glu + Val115Glu + Val138His + Ser141Glu  
Ser 70Glu + Ala114Ser + Thr137Ser + Ser207Asp + Ser216Asp  
Gly 69Asp + Gly110Asp + Thr137Glu + Pro169Ser + Tyr171His  
Ser 70Asp + Thr137Glu + Ala164His + Trp208Ile + Ser220Glu  
Ser 70Glu + Thr137Glu + Thr217Asn + Ser220Glu + Gly223Asn  
Gly110Gln + Trp112Cys + Val115Glu + Asn140Asp + Ser216Glu  
Asn 68Asp + Ser109Glu + Asn166Gln + Trp208Pro + Leu221Asp  
Thr113Pro + Thr167Glu + Tyr171Glu + Thr217Gln + Ser220Glu  
Trp112Ile + Leu134His + Gly139Asp + Gly162Glu + Trp208Val  
Gly110Asp + Gly162Gln + Trp208Ile + Tyr210Glu + Gly223Asp  
Gly 67Asp + Thr111Asp + Trp208Val + Tyr210Ser + Ser216Glu  
Gly 69Asn + Thr 73Glu + Asn106Asp + Ser141Glu + Gly162Asn  
Gly 69Glu + Gly135Asn + Pro169Asn + Trp208Ala + Thr217Glu  
Ala203Gly + Trp208Ala + Pro214Glu + Ala219Gln + Leu221Asp  
Thr 73Gly + Gly110Glu + Pro214Asp + Ser220Glu + Leu221Asn  
Val103Ala + Thr167Gly + Ser192Glu + Pro214Glu + Ser220Asp  
Asp105Glu + Tyr171Gln + Pro214Asp + Thr217Ser + Ser220Asp  
Tyr171Val + Ser191Asp + Trp208Pro + Pro214Glu + Ser220Glu  
Gly 67Asp + Gly162Ser + Gly206Ser + Ser207Glu + Thr217Glu  
Gly 67Glu + Trp112Phe + Ser207Asp + Thr217Asp + Ala219Gln  
Asn 68Asp + Asn163Glu + Phe193Met + Gly206Ser + Leu221Glu  
Asn 66Asp + Leu104Ala + Ser109Glu + Ala168Thr + Ser216Glu  
Val138Met + Gly139Asp + Gly162Ser + Ala168Glu + Ser220Asp

TABLE 36

### Multi-loop Sextuple Mutation Variants

Asn 66Gln + Gly 67Ser + Gly 69Ser + Leu104Gly +  
Tyr210Pro + Thr217Glu

Ser 70Asp + Gly110Pro + Gly136Gln + Phe193Ser +  
Trp208Cys + Leu221His  
Thr113Pro + Gly139Asn + Ser141Glu + Tyr171Pro +  
Trp208Thr + Leu221Gly  
Gly 69Gln + Leu104Thr + Ser107Asp + Leu134Thr +  
Thr215Ser + Leu221Gly  
Leu104Met + Leu134Ala + Thr137Asp + Trp208Gly +  
Tyr210Val + Pro214Gly  
Gly 69Ser + Thr 73Asp + Gly110Ser + Tyr210Leu +  
Thr215Gly + Thr217Ser  
Asn 66Ser + Gly 72Gln + Thr 73Pro + Gly110Asp +  
Trp208Val + Leu221His  
Thr111Asn + Ala164His + Thr167Glu + Phe193Met +  
Tyr210Asn + Thr217Gln  
Asn 66Gln + Thr 73Gly + Thr113Asp + Asn166Ser +  
Thr167Pro + Tyr210Pro  
Leu104Met + Pro205Gln + Ser207Asp + Trp208Ala +  
Thr212Gly + Thr217Pro  
Leu134Val + Gly165Gln + Trp208Thr + Pro214Gly +  
Leu221Ile + Thr224Asp  
Asn 66Ser + Ser109Asp + Ala114Asn + Val115Asn +  
Trp208Gln + Tyr210Ser  
Asn106Gln + Ala164Pro + Gly165Ser + Tyr171Ala +  
Ser220Glu + Thr224Ser  
Gly110Ser + Gly136Pro + Ile209His + Thr217Gln +  
Leu221Ile + Thr224Asp  
Asn 66Gln + Val115Pro + Trp208Asp + Thr217Ser +  
Leu221Ile + Thr224Pro  
Gly 67Glu + Thr 73Asn + Asn170Ser + Tyr210Ser +  
Thr212Gln + Gly223Pro  
Val103Gly + Ala164Thr + Tyr210His + Ala219His +  
Ser220Glu + Leu221Gly  
Thr 73Pro + Asn140Gln + Thr167Asp + Trp208Met +  
Thr217Gln + Leu221Asn  
Ser109Asp + Ala164His + Tyr210Pro + Thr212Ser +  
Gly223Pro + Thr224Asn  
Gly110Gln + Tyr210Pro + Tyr213Thr + Tyr218Met +  
Ser220Asp + Leu221His  
Val138Pro + Ala168Gly + Pro169Gln + Tyr210Ser +  
Thr217Gln + Ser220Asp  
Ala114Thr + Thr137Asn + Gly139Ser + Trp208Pro +  
Pro214Gln + Ser220Glu  
Val103Met + Thr137Ser + Ala203Ser + Tyr210Thr +  
Leu221Met + Gly223Ser  
Leu104Glu + Thr111Ser + Val115His + Gly165Asn +  
Tyr210Met + Ala219His  
Gly110Pro + Leu134Pro + Asn166Asp + Asn170Ser +  
Trp208Asn + Thr217Asn  
Thr167Glu + Pro169Gly + Asn170Gln + Trp208Gly +  
Thr215Ser + Leu221Ser  
Thr111Ser + Thr137Ser + Thr195Gln + Ser207Glu +  
Tyr210Val + Thr217Pro  
Asn106Asp + Gly136Pro + Gly162Ser + Trp208Ile +

Tyr210Gly + Pro214Gln  
Ser 70Asp + Gly165Gln + Phe193Asn + Thr195Ser +  
Trp208Pro + Thr212Ser  
Gly 72Ser + Ala114Gln + Asn166Gln + Phe193Glu +  
Tyr210Gly + Thr224Pro  
Asn 66Ser + Gly136Ser + Gly162Pro + Asn163Ser +  
Ala164Asp + Ala168Gln  
Gly 67Pro + Asn106Gln + Ser191Asp + Trp208His +  
Tyr218His + Leu221Asn  
Trp112Leu + Ala168Pro + Trp208His + Tyr210Ser +  
Thr217Gly + Ser220Asp  
Asn 66Ser + Thr111Gly + Val115Gly + Ala168Ser +  
Asn170Gln + Pro214Gly  
Gly110Ser + Trp112Val + Gly162Asn + Thr167Pro +  
Pro169Gln + Ser216Asp  
Gly 69Asn + Asn106Ser + Gly139Glu + Asn170Ser +  
Tyr210Cys + Leu221Gly  
Gly 69Asn + Ser141Asp + Phe193Asn + Tyr210Ile +  
Ala219Asn + Leu221Ser  
Ser109Asp + Pro169Ser + Trp208His + Pro214Asn +  
Thr215Asn + Thr217Gly  
Asn 68Asp + Gly162Gln + Ala164His + Trp208Pro +  
Tyr210Leu + Leu221Val  
Val103Thr + Gly139Pro + Ala164Thr + Thr217Ser +  
Leu221Pro + Thr224Pro  
Gly 69Glu + Gly 72Gln + Thr137Pro + Trp208Ile +  
Tyr218Pro + Gly223Gln  
Gly 67Pro + Leu104Cys + Val138Met + Gly139Ser +  
Ala203Gln + Ala219Asn  
Trp112Ser + Phe193Ala + Thr217Gly + Tyr218Glu +  
Leu221Met + Thr224Asn  
Asn163Gln + Trp208Leu + Tyr210Ser + Thr217Glu +  
Ala219Gly + Gly223Pro  
Val103His + Gly110Gln + Gly139Ser + Ser192Asp +  
Phe193Asn + Thr217Gln  
Gly108Gln + Pro169Asn + Thr195Ser + Trp208Ala +  
Thr215Pro + Thr217Glu  
Ser109Asp + Thr111Asn + Trp112Gln + Thr167Gln +  
Thr195Gly + Tyr210Asn  
Gly 72Gln + Trp112Leu + Gly136Asp + Tyr171His +  
Tyr210Thr + Leu221Ser  
Gly 69Glu + Ala164Pro + Trp208Val + Tyr210Thr +  
Tyr218Gln + Thr224Ser  
Ala114Pro + Leu134Glu + Asn140Ser + Ala164Gln +  
Phe193Ile + Trp208Asn  
Asn 66Ser + Val138Met + Gly139Asn + Asn163Gln +  
Gly165Gln + Tyr210Thr  
Asn106Gln + Ser191Asp + Phe193Leu + Trp208Val +  
Pro214Gln + Thr217Ser  
Ala114Thr + Asn166Ser + Tyr171Gly + Ser191Glu +  
Ala203Pro + Tyr210Ala  
Asn 68Gln + Tyr171Asp + Phe193Tyr + Gly206Ser +  
Tyr210Val + Leu221Thr

Leu104Ala + Ala114Ser + Asn163Asp + Thr195Gly +  
Gly206Gln + Leu221Ala  
Gly110Asn + Val115Asn + Trp208Cys + Tyr210His +  
Ala219Thr + Gly223Asp  
Asn 66Asp + Gly 69Asn + Val115Ala + Ala164Ser +  
Trp208Gln + Thr217Gln  
Val115Met + Thr137Ser + Gly162Asn + Asn166Glu +  
Tyr171His + Pro214Ser  
Asn106Gln + Gly110Gln + Trp208His + Thr217Pro +  
Ala219Asp + Ser220Asp  
Gly 67Asn + Gly 72Asn + Ala114Gly + Asn140Gln +  
Ser216Asp + Thr217Asp  
Gly 69Gln + Thr 73Ser + Pro169Gln + Trp208Ile +  
Ser220Glu + Leu221Asp  
Asn170Glu + Tyr171Asp + Phe193Ser + Trp208His +  
Leu221Thr + Thr224Gly  
Gly 67Ser + Gly 69Glu + Ser 70Glu + Val103Gly +  
Tyr210Gln + Ala219Asn  
Trp112Gly + Asn166Ser + Trp208Met + Tyr210Glu +  
Ser220Glu + Thr224Asn  
Leu134Pro + Thr195Gly + Trp208Glu + Tyr210Asp +  
Thr217Ser + Ser220Glu  
Leu104Gln + Trp112Gln + Tyr171Pro + Trp208Glu +  
Thr217Gly + Leu221Glu  
Asp105Glu + Gly108Asp + Gly136Gln + Thr167Gln +  
Tyr171Gln + Leu221Val  
Ser107Glu + Ser109Glu + Val138Met + Ala168His +  
Pro169Gln + Phe193Ser  
Gly 67Glu + Ser 70Glu + Trp112His + Tyr171Ile +  
Trp208His + Tyr210Ala  
Val103Pro + Trp208Glu + Tyr210Ser + Ala219Glu +  
Ser220Glu + Ser222Asp  
Ser191Asp + Ser207Glu + Tyr210Ala + Thr215Gly +  
Thr217Gln + Gly223Ser  
Asn106Glu + Ser109Asp + Ala168Thr + Tyr171Met +  
Phe193Ile + Trp208Leu  
Asn 68Ser + Thr137Gln + Ser191Asp + Ser207Asp +  
Trp208Asp + Leu221Thr  
Val103Gln + Val138His + Asn166Asp + Ser192Glu +  
Trp208His + Leu221Gly  
Val103Pro + Gly139Pro + Asn163Asp + Gly165Gln +  
Ala168Glu + Tyr210Leu  
Asn 68Ser + Thr113Gln + Leu134Ile + Ser191Glu +  
Leu221His + Gly223Glu  
Gly110Gln + Ser207Glu + Trp208Gln + Thr217Pro +  
Tyr218Gly + Ser220Asp  
Gly162Ser + Phe193Ala + Trp208Glu + Ile209Ser +  
Leu221Cys + Gly223Glu  
Ser 70Asp + Asp105Glu + Gly108Glu + Asn140Gln +  
Trp208Leu + Thr217Gly  
Gly110Asn + Thr137Glu + Pro169Asp + Asn170Ser +  
Thr195Glu + Tyr210His  
Asn 68Gln + Gly162Glu + Ala164Thr + Gly165Asp +



Tyr171Ala + Ser192Asp  
Val138Met + Gly139Asn + Asn170Gln + Tyr210Glu +  
Thr217Asp + Ser220Glu  
Gly139Ser + Asn163Asp + Ala164Ser + Ser191Asp +  
Tyr210Gly + Leu221Ala  
Ser 70Asp + Thr 73Asp + Thr167Ser + Trp208Ser +  
Thr217Asp + Ala219Asp  
Asn 66Glu + Leu104Glu + Asp105Glu + Ser107Asp +  
Leu221Ser + Gly223Pro  
Asn 66Glu + Thr 73Asp + Gly110Pro + Ala168Gly +  
Tyr218Val + Leu221Ile  
Gly 67Asn + Asp105Glu + Asn106Glu + Thr111Glu +  
Gly165Asn + Thr217Gly  
Asn 66Asp + Tyr210Ser + Pro214Glu + Thr215Asp +  
Thr217Gln + Tyr218Asp  
Thr 73Asn + Ser192Glu + Ala203Thr + Ser207Asp +  
Trp208Glu + Leu221Glu  
Val138Gly + Pro169Glu + Ser192Glu + Thr195Glu +  
Trp208Pro + Thr212Gln  
Thr 73Asp + Gly108Asn + Leu134Ser + Tyr210Ala +  
Thr217Gln + Tyr218Glu  
Thr 73Asn + Ala114Thr + Val138Ser + Gly139Asp +  
Ser141Glu + Asn170Glu  
Gly108Gln + Gly135Pro + Gly162Asn + Ser220Glu +  
Leu221Ala + Gly223Glu  
Val103Gln + Trp112Cys + Ala164Glu + Trp208Gln +  
Thr217Gly + Gly223Asp  
Thr111Gln + Leu134His + Ser191Asp + Phe193Asp +  
Thr195Gln + Trp208Asp  
Val103Ser + Leu104Ile + Gly110Glu + Gly136Glu +  
Ala164Asn + Tyr210Gln  
Leu134Ser + Thr167Gly + Ser191Glu + Ser220Asp +  
Leu221Pro + Ser222Asp  
Gly 69Gln + Trp208Asp + Pro214Glu + Ser220Asp +  
Ser222Glu + Gly223Asn  
Ser107Asp + Ser109Glu + Trp112Pro + Gly135Asp +  
Ala168Thr + Thr212Gly  
Asn140Ser + Thr167Asp + Trp208Glu + Thr217Asn +  
Ser220Asp + Leu221Asp  
Gly135Asp + Gly162Glu + Tyr171Asp + Tyr210Ala +  
Thr217Ser + Ser222Asp  
Val115Asp + Tyr210Gln + Ser220Glu + Leu221Asp +  
Ser222Asp + Gly223Ser  
Ala114His + Asn166Asp + Tyr171Cys + Tyr210Glu +  
Ser220Asp + Leu221Asp  
Val115Cys + Gly139Asn + Ala164Gly + Pro205Asn +  
Thr217Asp + Ser220Asp  
Thr167Gln + Tyr210Ile + Thr215Gly + Thr217Asp +  
Tyr218Asn + Ser220Asp  
Thr137Ser + Trp208Leu + Tyr210Leu + Thr217Glu +  
Ser220Asp + Leu221Gly  
Thr111Asn + Gly135Ser + Trp208Asp + Tyr210Glu +  
Thr217Glu + Leu221Ala

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Thr113Asn + Ala114Gly + Thr137Asp + Thr167Asp +  
Trp208Gln + Gly223Asn  
Ser 70Asp + Thr 73Glu + Asn163Asp + Thr167Pro +  
Pro214Glu + Thr217Gly  
Gly135Glu + Tyr171Asn + Pro205Gln + Ser207Glu +  
Trp208Glu + Ser220Asp  
Gly 69Asp + Asp105Glu + Gly108Asp + Ser207Asp +  
Trp208Thr + Gly223Asn  
Thr113Gly + Ala114Pro + Tyr210Asp + Pro214Asp +  
Thr217Gln + Ser220Asp  
Asn 68Glu + Ser 70Asp + Asp105Glu + Ala114Thr +  
Gly135Ser + Ser220Asp  
Thr111Ser + Thr137Glu + Ser207Glu + Trp208Thr +  
Ser220Glu + Leu221Asp  
Gly 69Asp + Leu104Asp + Asp105Glu + Asn140Glu +  
Asn166Ser + Leu221His  
Thr137Asp + Gly139Glu + Asn170Glu + Trp208Cys +  
Tyr210Asp + Leu221Thr  
Gly 67Pro + Trp112Gln + Ser192Asp + Tyr210Ile +  
Ser220Asp + Ser222Asp  
Val103Gln + Gly108Asp + Gly135Glu + Thr137Glu +  
Ala168Gln + Tyr210Asn  
Val115Asp + Leu134Glu + Asn166Gln + Asn170Asp +  
Tyr210Val + Thr212Pro  
Asn 66Asp + Asn 68Ser + Thr167Gln + Ile209Leu +  
Ser216Asp + Thr217Glu  
Val103Glu + Gly108Asp + Gly110Glu + Gly165Pro +  
Thr195Glu + Thr217Gln  
Asn 66Asp + Ser 70Glu + Thr 73Asp + Ser109Asp +  
Gly136Pro + Tyr210Ile  
Gly 69Asp + Ser 70Glu + Asp105Glu + Asn163Glu +  
Tyr210Met + Thr217Gln  
Asn 68Gln + Leu134Glu + Ser207Asp + Trp208Pro +  
Tyr210Asp + Leu221Asp  
Asn 66Ser + Val115Gly + Gly162Asp + Ser191Glu +  
Trp208Ile + Thr217Ser  
Ser 70Glu + Tyr213Thr + Thr217Asn + Tyr218Gly +  
Ser222Glu + Gly223Asp  
Trp112Met + Gly165Asp + Pro169Ser + Ser207Glu +  
Trp208Gly + Ser222Asp  
Gly 69Ser + Ser109Asp + Gly110Glu + Ala114Gly +  
Gly136Asp + Phe193Asp  
Ala164Gln + Gly165Pro + Ser192Asp + Phe193Asp +  
Ser207Glu + Thr217Asp  
Asn 68Glu + Thr 73Asn + Gly162Gln + Trp208Gln +  
Ala219Glu + Ser220Asp  
Ser 70Glu + Leu134Ser + Gly165Asp + Asn166Asp +  
Ala168Gly + Ile209Gly  
Leu104Asp + Asp105Glu + Trp208Gly + Tyr210Glu +  
Tyr218Ala + Gly223Pro  
Asn 68Asp + Gly 69Glu + Thr111Asn + Trp112His +  
Gly136Asn + Pro169Glu  
Gly108Ser + Asn140Asp + Ser141Glu + Ala164Gln +

Ser207Glu + Tyr218Ser  
Leu104Cys + Gly162Pro + Ser207Glu + Ser216Glu +  
Thr217Asp + Ala219Thr  
Ala164Asn + Asn166Asp + Gly206Gln + Tyr210Thr +  
Ser216Asp + Thr217Asp  
Gly108Asp + Ser109Asp + Asn166Ser + Thr217Gln +  
Ser220Asp + Leu221Thr  
Ser109Asp + Gly110Asp + Val138Ala + Tyr171His +  
Ala204His + Ser222Glu  
Asn 68Gln + Thr137Gly + Pro169Asp + Ser207Glu +  
Trp208Glu + Tyr218Gln  
Trp112Asp + Thr113Glu + Val138Gly + Ser191Glu +  
Trp208Pro + Leu221Ser  
Leu104Gly + Asp105Glu + Asn106Glu + Gly206Ser +  
Trp208His + Tyr218Glu  
Gly108Asp + Leu134Asn + Gly136Gln + Trp208Phe +  
Ser220Asp + Leu221Asp  
Gly108Gln + Trp112Tyr + Asn170Glu + Trp208Gln +  
Ser220Glu + Leu221Glu  
Gly 72Asn + Asn166Asp + Phe193Ser + Trp208Tyr +  
Ser220Glu + Leu221Glu  
Asn 66Glu + Gly 67Glu + Thr111Asn + Thr215Gln +  
Thr217Asn + Ser220Glu  
Asn106Asp + Ser107Asp + Gly165Asp + Phe193Val +  
Trp208Val + Leu221Thr  
Leu104Val + Gly165Gln + Ser192Asp + Phe193Glu +  
Tyr210Glu + Leu221Cys  
Gly136Gln + Pro169Ser + Ser192Asp + Phe193Asp +  
Trp208Leu + Ser220Glu  
Ser107Asp + Val115Thr + Thr137Gln + Gly162Asp +  
Asn163Asp + Ala203His  
Val103Met + Ser107Glu + Trp208Val + Thr215Glu +  
Ser216Glu + Gly223Ser  
Val103Ser + Asn140Asp + Ser141Glu + Tyr210Glu +  
Ser220Asp + Gly223Asn  
Asn163Asp + Asn170Glu + Thr195Asn + Ser207Asp +  
Tyr210Met + Tyr218Pro  
Asn 66Asp + Gly139Gln + Tyr210Glu + Thr217Ser +  
Ser220Asp + Thr224Asn  
Gly 69Asp + Asn106Asp + Gly110Asp + Gly165Asn +  
Pro169Ser + Thr217Asp  
Phe193Ile + Thr215Ser + Thr217Glu + Ser220Asp +  
Leu221Pro + Gly223Glu  
Val103Gly + Asp105Glu + Thr195Asn + Ser207Asp +  
Ser222Asp + Gly223Asn  
Gly 69Asp + Asn140Gln + Ala164His + Ala168Ser +  
Ser207Asp + Ser222Asp  
Asn 68Asp + Thr111Asn + Ala114Gly + Gly135Gln +  
Ser207Glu + Ser222Glu  
Asn106Asp + Gly139Asn + Gly162Glu + Pro169Ser +  
Ser191Asp + Thr224Asp  
Thr113Asp + Ser192Asp + Ser207Glu + Tyr213Thr +  
Thr215Pro + Ser222Asp

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Gly 69Pro + Asn163Asp + Thr195Glu + Trp208Ser +  
Tyr210Glu + Leu221Cys  
Gly 69Pro + Ser 70Asp + Gly110Ser + Gly135Glu +  
Asn170Glu + Leu221Ile  
Gly 69Glu + Ala164His + Thr195Gln + Ser220Glu +  
Leu221Glu + Thr224Glu  
Gly 69Gln + Gly136Asp + Val138Asp + Pro169Gly +  
Asn170Gln + Ser220Glu  
Gly136Glu + Val138Asp + Thr195Asn + Ser220Glu +  
Leu221Gly + Gly223Pro  
Asn 68Gln + Ser107Glu + Ser109Glu + Gly139Gln +  
Ala164Gln + Ser207Asp  
Ser107Glu + Ser109Glu + Pro169Gln + Tyr210Met +  
Thr217Pro + Ser222Asp  
Gly 67Asp + Ser 70Glu + Val103Ala + Thr111Glu +  
Tyr210Asn + Pro214Ser  
Asn 66Glu + Gly 67Asp + Leu134His + Ser191Asp +  
Ser207Glu + Trp208Thr  
Asn 66Glu + Val103Asp + Gly108Asn + Asn170Ser +  
Thr217Asn + Leu221Met  
Thr 73Glu + Trp112Pro + Asn166Ser + Phe193His +  
Tyr218Ile + Ser220Glu  
Val115His + Ser207Glu + Trp208Tyr + Tyr210Leu +  
Pro214Glu + Leu221Glu  
Asn 66Gln + Ser107Glu + Ser109Glu + Ser207Glu +  
Gly223Asp + Thr224Gly  
Asn 66Glu + Asn 68Asp + Thr 73Gln + Thr195Ser +  
Ser207Asp + Tyr218Pro  
Ser 70Asp + Leu104Ile + Asn163Asp + Thr217Gln +  
Leu221Val + Thr224Glu  
Gly 67Asp + Val103Glu + Asn106Asp + Ala168Asn +  
Thr195Glu + Trp208Leu  
Thr137Gly + Gly162Glu + Phe193Asp + Trp208Asp +  
Thr217Asn + Tyr218Val  
Gly135Pro + Gly139Glu + Ser207Glu + Trp208Ala +  
Tyr210Cys + Gly223Glu  
Asn106Ser + Thr137Asp + Gly139Glu + Ser141Asp +  
Ala204Gln + Trp208Glu  
Trp112Ala + Asn163Glu + Ser192Glu + Trp208Val +  
Tyr210Glu + Ser220Glu  
Gly110Asp + Trp112Asp + Tyr210Thr + Ser220Glu +  
Leu221Met + Ser222Glu  
Gly 67Asp + Gly 69Asp + Thr 73Pro + Ser109Asp +  
Trp208Gln + Ser222Asp  
Asn 66Ser + Val103Glu + Asp105Glu + Trp112His +  
Ser191Glu + Tyr210Ala  
Gly 67Glu + Gly 69Asn + Gly162Glu + Ser207Glu +  
Thr217Asn + Gly223Glu  
Ser 70Asp + Gly165Asp + Ala168Asp + Trp208Pro +  
Thr217Gly + Leu221Ile  
Gly 67Glu + Val103Asn + Gly136Gln + Trp208Pro +  
Ser216Glu + Leu221Met  
Gly 67Asn + Gly139Glu + Ser141Glu + Tyr213Gly +

Ser220Asp + Leu221Asn  
Gly 67Glu + Leu104Ser + Tyr210Ser + Ser220Asp +  
Ser222Glu + Gly223Gln  
Gly 67Asp + Asn 68Glu + Gly136Asp + Ala168Gln +  
Pro169Glu + Phe193Asn  
Asn 66Glu + Gly 69Asp + Asn140Gln + Ser192Asp +  
Phe193Asp + Tyr210Leu  
Leu104Asp + Asn106Glu + Asn170Glu + Tyr210Ile +  
Thr215Ser + Thr224Pro  
Leu104Asp + Ser107Glu + Gly135Asp + Gly139Asn +  
Ala164Glu + Thr217Gly  
Trp112Glu + Gly139Glu + Ala164Glu + Thr195Glu +  
Thr212Ser + Tyr213Ser  
Asn 68Asp + Thr111Gly + Thr113Ser + Val138Asp +  
Asn170Asp + Phe193Ser  
Asn163Gln + Ala168Asp + Asn170Asp + Ser207Glu +  
Thr217Gly + Ser220Asp  
Gly 67Asp + Asn106Glu + Thr113Ser + Gly136Gln +  
Asn166Glu + Leu221Cys  
Asn 66Gln + Gly110Asp + Ala114His + Gly136Glu +  
Thr137Asp + Ser222Glu  
Gly 67Asp + Asn 68Ser + Gly 72Pro + Gly139Ser +  
Thr167Glu + Thr195Asp  
Asn 66Asp + Asn 68Gln + Ser191Glu + Ser207Asp +  
Trp208Val + Ser220Asp  
Ser109Glu + Ala114Glu + Leu134Ile + Gly136Ser +  
Ala219Glu + Ser220Asp  
Val115Glu + Ala168His + Pro169Asn + Tyr210Asp +  
Ser216Glu + Ala219Asp  
Asn 66Glu + Asn106Asp + Asn170Ser + Trp208Thr +  
Ser216Asp + Tyr218Gly  
Gly162Asn + Asn170Asp + Phe193Glu + Tyr210Gln +  
Ser220Glu + Thr224Asp  
Gly108Ser + Leu134Pro + Thr137Asp + Pro169Glu +  
Ser207Glu + Thr217Gly  
Thr137Asp + Val138Glu + Asn163Glu + Ala164Asn +  
Ala168Ser + Thr217Glu  
Gly162Glu + Trp208Glu + Tyr210Val + Thr217Glu +  
Ser220Glu + Gly223Pro  
Gly135Asp + Val138Thr + Ser207Asp + Thr217Pro +  
Tyr218Ile + Ser220Glu  
Leu104Gly + Ser192Asp + Thr195Asp + Thr217Pro +  
Ser220Glu + Leu221Asn  
Asn 66Ser + Gly136Glu + Val138Glu + Trp208Leu +  
Thr217Asp + Ser220Glu  
Gly 69Ser + Val103Asp + Ser109Asp + Ser191Asp +  
Gly206Gln + Tyr210Pro  
Ala114Gly + Gly162Asp + Pro214Gly + Thr215Asp +  
Thr217Glu + Ser220Glu  
Thr137Gln + Gly139Asp + Gly165Pro + Tyr171Glu +  
Thr217Glu + Ser220Glu  
Gly 67Glu + Asp105Glu + Asn140Glu + Phe193Gly +  
Trp208Leu + Leu221Cys

Leu104Glu + Trp112Leu + Phe193Leu + Ser207Asp +  
Trp208Ala + Thr224Asp  
Ser 70Glu + Thr113Gly + Gly162Ser + Asn163Asp +  
Ala164Glu + Ser220Asp  
Gly 69Glu + Ser 70Asp + Thr111Glu + Ser220Asp +  
Leu221Pro + Gly223Ser  
Asn 66Gln + Leu104Pro + Gly136Glu + Asn163Asp +  
Tyr171Ile + Leu221Glu  
Ser 70Asp + Leu104Glu + Ala168Asn + Pro169Glu +  
Thr195Asp + Trp208Phe  
Ser 70Glu + Gly162Glu + Trp208Glu + Thr217Gly +  
Leu221Thr + Ser222Asp  
Gly 69Asn + Thr137Glu + Gly139Pro + Asn166Asp +  
Pro169Asp + Trp208Asp  
Gly 69Glu + Leu104Glu + Val115Ser + Asn140Ser +  
Ser192Glu + Pro214Asp  
Leu104His + Asp105Glu + Val138His + Asn163Asp +  
Ser192Glu + Thr217Asn  
Ala114Asn + Asn163Glu + Asn170Ser + Ser192Asp +  
Ala204Asn + Thr217Asp  
Asn 68Gln + Thr 73Asp + Ser109Glu + Trp208Phe +  
Pro214Glu + Ser220Asp  
Val115Cys + Leu134Asp + Gly135Pro + Ala164Thr +  
Asn170Asp + Trp208Asp  
Asn 66Glu + Gly 69Glu + Trp112Gln + Gly135Asn +  
Gly139Asp + Asn170Glu  
Thr 73Gly + Ser107Glu + Gly135Asp + Pro169Asp +  
Trp208Thr + Leu221Asn  
Gly 67Ser + Ser 70Glu + Gly136Asp + Pro169Asn +  
Leu221Asp + Gly223Asp  
Ser 70Asp + Gly110Glu + Val138Ser + Tyr210Val +  
Leu221Asp + Gly223Asp  
Asn 66Asp + Leu104Glu + Ser109Glu + Tyr210Asn +  
Thr217Asn + Ser220Asp  
Asn 66Glu + Gly 67Asp + Asn163Glu + Ser207Glu +  
Thr215Gly + Leu221Thr  
Gly 69Glu + Tyr210Glu + Thr215Asp + Thr217Gln +  
Tyr218Gln + Ser220Glu  
Ser 70Asp + Thr137Glu + Ala164His + Thr167Gly +  
Trp208Asp + Ser220Glu  
Val138Met + Gly139Gln + Asn140Glu + Ala168Pro +  
Tyr171Glu + Thr215Glu  
Val103Gly + Asn106Ser + Ser107Asp + Thr111Asp +  
Ser222Glu + Gly223Glu  
Gly 72Gln + Asn106Glu + Gly110Asp + Thr113Glu +  
Gly135Asn + Ala219Glu  
Ser109Glu + Thr113Asp + Trp208Ile + Ser220Glu +  
Leu221Asp + Thr224Ser  
Gly 67Asn + Ser 70Asp + Gly108Asp + Asn170Glu +  
Thr215Pro + Leu221Thr  
Gly 67Asp + Ser107Asp + Thr167Gly + Tyr210Asn +  
Ser220Asp + Ser222Glu  
Gly 69Asp + Thr 73Glu + Asn106Gln + Thr111Asn +

Val138Glu + Thr217Asp  
Thr111Asn + Ser191Asp + Thr195Asp + Ala204Thr +  
Ala219Ser + Ser220Glu  
Ser109Asp + Ser191Glu + Tyr210Val + Thr215Asn +  
Leu221Gly + Ser222Glu  
Thr 73Glu + Leu104Asp + Asn106Gln + Thr195Ser +  
Ser207Glu + Ser220Asp  
Asn 66Asp + Thr 73Asp + Leu104Glu + Gly136Gln +  
Gly162Asp + Leu221Gly  
Gly 72Asn + Leu104Asp + Asn163Asp + Tyr171Ser +  
Ser191Glu + Thr217Asn  
Val115Asp + Gly136Gln + Tyr210Glu + Tyr218Cys +  
Leu221Asn + Ser222Asp  
Thr 73Asp + Asn140Ser + Asn163Glu + Thr195Asp +  
Ser220Asp + Leu221Pro  
Asn 68Glu + Leu104Asp + Pro169Asn + Trp208Val +  
Thr217Asn + Gly223Glu  
Gly110Glu + Thr167Gln + Ser192Glu + Ser207Glu +  
Trp208Cys + Ser220Asp  
Val103Gly + Gly136Glu + Asn140Asp + Ser207Asp +  
Tyr213Asn + Ser220Asp  
Thr113Asp + Gly136Asp + Thr137Gln + Asn163Glu +  
Asn166Glu + Trp208Phe  
Asn 66Glu + Gly 69Glu + Ser109Asp + Ser192Asp +  
Phe193Val + Tyr218Ala  
Ser 70Glu + Ser141Asp + Asn163Ser + Trp208Met +  
Tyr210Pro + Thr215Asp  
Asp105Glu + Gly110Asn + Thr111Glu + Leu134Ala +  
Ser141Glu + Phe193Tyr  
Asn 66Glu + Gly 69Ser + Gly108Asp + Thr111Asp +  
Thr195Gly + Tyr210Leu  
Gly165Glu + Tyr171Asn + Ser207Glu + Thr215Glu +  
Thr217Gly + Thr224Glu  
Asn 66Gln + Thr 73Ser + Gly139Asn + Pro214Asp +  
Ser220Asp + Leu221Ile  
Asn166Glu + Ser192Asp + Trp208Thr + Tyr210Gly +  
Thr215Asp + Ser222Glu  
Asn 66Asp + Ser191Asp + Thr215Asn + Ala219Asp +  
Ser222Asp + Gly223Gln  
Asp105Glu + Gly108Glu + Thr195Asn + Ile209Leu +  
Pro214Asp + Ser220Asp  
Thr111Glu + Val138Asp + Asn163Ser + Tyr218Met +  
Leu221Glu + Gly223Asp  
Gly 67Glu + Val138Glu + Ser141Glu + Trp208Ile +  
Pro214Asn + Thr215Asp  
Trp112Leu + Thr113Gln + Gly165Glu + Thr167Asp +  
Thr217Glu + Ser220Asp  
Gly 67Gln + Asn 68Asp + Gly 69Asp + Phe193Ala +  
Ser216Glu + Ser222Glu  
Ser 70Asp + Val115Glu + Gly162Ser + Ala168Thr +  
Tyr210Gly + Thr217Asp  
Ser 70Asp + Gly136Asn + Gly165Glu + Tyr210Asn +  
Thr217Asp + Leu221His

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Thr 73Ser + Val103Asn + Ser109Asp + Ser192Asp +  
Ser207Asp + Trp208Ile  
Asn106Asp + Ser192Glu + Phe193Pro + Ser207Glu +  
Thr217Gln + Tyr218Ile  
Ser141Asp + Thr167Asp + Ala168Asp + Ser191Asp +  
Ala219Asn + Leu221Thr  
Asn 66Gln + Ser141Asp + Asn170Ser + Thr217Gln +  
Ser220Asp + Gly223Asp  
Thr 73Gly + Asp105Glu + Ala114His + Ser220Glu +  
Leu221Asn + Gly223Glu  
Asn106Asp + Asn140Gln + Trp208Ser + Tyr210Cys +  
Ser220Asp + Gly223Glu  
Gly 67Gln + Asp105Glu + Ala164Asp + Pro169Asn +  
Tyr218Leu + Gly223Asp  
Trp112Val + Asn166Asp + Phe193Gly + Pro214Glu +  
Ser220Asp + Ser222Glu  
Gly108Asp + Thr111Asn + Ser192Glu + Thr217Asn +  
Ala219Glu + Ser220Asp  
Val103Ser + Val115Asp + Gly139Glu + Asn166Ser +  
Ser207Glu + Ser220Glu  
Leu104Asp + Asp105Glu + Asn140Glu + Thr167Asp +  
Pro214Gly + Ala219Gly  
Gly 67Asp + Asn 68Asp + Thr111Asp + Ala164Gln +  
Phe193Ser + Ser207Asp  
Ser109Glu + Trp208Asp + Ser216Glu + Thr217Asp +  
Tyr218Asn + Leu221His  
Ser141Asp + Ala168Glu + Tyr210Met + Ser216Glu +  
Thr217Asp + Tyr218Ala  
Ser109Asp + Gly110Asp + Asn166Asp + Asn170Ser +  
Thr215Ser + Leu221Asp  
Gly110Glu + Thr167Glu + Ala168Asp + Trp208Met +  
Ser220Asp + Thr224Gln  
Asp105Glu + Asn106Asp + Gly165Asp + Thr195Ser +  
Tyr218Ile + Ser220Asp  
Asn106Glu + Ser141Asp + Tyr218Ser + Ala219Thr +  
Ser220Asp + Leu221Glu  
Thr 73Gln + Thr111Glu + Asn166Asp + Trp208Ser +  
Ser220Asp + Leu221Asp  
Ser107Glu + Ala168Asn + Pro169Asn + Ser191Asp +  
Ser192Asp + Thr217Asp  
Ala114Glu + Asn166Ser + Thr212Pro + Thr217Glu +  
Tyr218Asp + Thr224Asp  
Asn106Glu + Ser107Asp + Gly139Asp + Ala203Asn +  
Thr217Ser + Ser220Asp  
Thr113Asn + Gly139Ser + Ser141Glu + Thr215Glu +  
Ser216Asp + Ser220Asp  
Gly 69Gln + Asn140Glu + Gly162Glu + Asn163Asp +  
Tyr210Gln + Ser220Asp  
Asn 68Asp + Ala114Pro + Gly135Glu + Trp208Asp +  
Leu221Ile + Gly223Asp  
Asn 68Asp + Ala164Glu + Trp208Cys + Tyr210Glu +  
Ala219Thr + Ser220Asp  
Thr111Asp + Asn166Asp + Asn170Gln + Tyr210Asp +



Thr212Ser + Ser220Glu  
Ser141Glu + Gly165Asp + Trp208Ile + Tyr210Asp +  
Thr217Gln + Ser220Asp  
Asn 66Gln + Val103Asp + Gly108Pro + Thr137Glu +  
Tyr210Glu + Ser220Glu  
Gly108Glu + Thr137Gln + Asn163Glu + Tyr210Glu +  
Ser220Asp + Leu221Cys  
Gly 69Gln + Ser 70Glu + Gly135Glu + Asn163Asp +  
Thr217Gly + Ser220Asp  
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Thr195Asn + Leu221His  
Asn106Gln + Asn166Asp + Ala168Asn + Asn170Glu +  
Ser207Glu + Ser220Asp  
Asn 66Asp + Ser 70Asp + Gly110Gln + Gly136Asp +  
Pro214Gln + Ser220Asp  
Asn 68Ser + Pro169Asp + Ser192Glu + Ala219Asp +  
Gly223Ser + Thr224Asp  
Thr 73Pro + Gly135Ser + Phe193Glu + Gly206Gln +  
Tyr210Asp + Thr217Glu  
Trp112Glu + Val115Gln + Asn170Gln + Thr217Asp +  
Tyr218Met + Ser220Asp  
Gly110Asn + Val115Glu + Thr137Gln + Ile209Gly +  
Thr217Asp + Ser220Glu  
Gly108Glu + Thr113Gln + Pro214Asn + Thr217Glu +  
Tyr218Gln + Ser220Asp  
Gly110Ser + Thr111Gly + Thr113Glu + Phe193Pro +  
Thr217Glu + Ser220Asp  
Asn166Glu + Trp208Ile + Tyr210Met + Thr217Glu +  
Tyr218Val + Ser220Asp  
Ser 70Glu + Val103Glu + Val115Gln + Pro169Ser +  
Tyr210Ala + Ser220Asp  
Asn 66Asp + Ser107Asp + Trp208Met + Tyr210Pro +  
Ser220Asp + Ser222Glu  
Gly110Ser + Thr111Asp + Gly135Gln + Gly136Asp +  
Thr137Asn + Trp208Glu  
Thr167Asp + Ala168Ser + Pro169Glu + Trp208Thr +  
Thr215Asp + Leu221Asp  
Val115Gln + Ala168Gln + Pro169Gln + Phe193Asp +  
Tyr210Ser + Leu221Asp  
Asn 66Glu + Gly136Pro + Asn140Gln + Ser191Asp +  
Trp208Asp + Leu221His  
Gly136Glu + Gly139Glu + Ser192Asp + Ala203Asn +  
Trp208Asp + Leu221His  
Gly 67Asn + Thr113Gln + Thr137Glu + Thr167Asp +  
Ala219Gln + Leu221Glu  
Gly 69Asn + Thr 73Asp + Thr111Pro + Asn166Asp +  
Ser191Asp + Leu221Val  
Val138Pro + Asn166Glu + Ser191Asp + Tyr218Asn +  
Ser220Asp + Leu221Gln  
Asp105Glu + Gly139Asn + Gly165Glu + Trp208Met +  
Tyr218Thr + Gly223Asp  
Gly110Asn + Thr111Pro + Ala114Asp + Asn170Asp +  
Trp208Asp + Tyr210Asp

Gly 67Gln + Gly 69Glu + Ser107Asp + Thr167Glu +  
Ala168Asn + Ser207Asp  
Gly 69Glu + Gly165Glu + Trp208Cys + Tyr210Cys +  
Pro214Asp + Ser220Asp  
Ser 70Glu + Gly 72Gln + Val103Thr + Ser109Glu +  
Thr113Glu + Ser220Asp  
Gly 69Pro + Val115Glu + Tyr210Asn + Ser216Asp +  
Ser220Glu + Ser222Glu  
Gly136Glu + Trp208Ser + Ser216Asp + Ser220Asp +  
Leu221Asn + Ser222Asp  
Leu104Glu + Val115Pro + Gly162Ser + Asn166Asp +  
Ser220Asp + Ser222Asp  
Gly 67Glu + Val138Asp + Thr217Ser + Ser220Asp +  
Leu221His + Ser222Asp  
Asn 68Asp + Asn170Glu + Gly206Asn + Ser207Asp +  
Trp208Ile + Leu221Asp  
Asn106Gln + Ser109Glu + Thr113Asp + Leu134Gln +  
Thr137Glu + Trp208Asn  
Asn106Glu + Gly139Gln + Thr167Glu + Pro169Gln +  
Ser191Asp + Ser207Glu  
Ser107Glu + Val115Asp + Gly165Asn + Trp208Thr +  
Tyr218Asp + Ser220Glu  
Gly 72Asn + Asn106Gln + Gly139Asp + Pro169Glu +  
Trp208Pro + Ser220Glu  
Val138Asp + Ser141Glu + Pro169Ser + Ser207Glu +  
Ala219Glu + Leu221Thr  
Asn106Gln + Ala114Asp + Gly135Asp + Thr195Asn +  
Ser207Glu + Ser220Asp  
Ser 70Asp + Asn166Glu + Asn170Gln + Ser216Glu +  
Thr217Gln + Thr224Gly  
Gly 69Asp + Asn140Ser + Gly162Pro + Trp208Gly +  
Thr217Glu + Leu221Asp  
Asn 68Asp + Ser107Asp + Ala114Gln + Gly162Asp +  
Thr217Pro + Ser220Glu  
Ser 70Asp + Thr111Asp + Ile209Met + Thr217Gly +  
Ser220Glu + Leu221Asn  
Ser 70Asp + Leu104His + Thr111Gln + Tyr171Ser +  
Phe193Asp + Ser220Glu  
Thr113Gly + Gly139Pro + Ser141Glu + Tyr171Glu +  
Ser192Asp + Ile209Ser  
Trp112Ala + Thr113Glu + Val138Gln + Ser141Asp +  
Ser216Asp + Leu221Ser  
Gly 69Asn + Asn106Asp + Trp112Gly + Gly162Asp +  
Phe193Glu + Ser220Glu  
Asn 68Ser + Val103Glu + Asn106Asp + Ala168Pro +  
Pro169Asp + Pro214Glu  
Leu104Glu + Ala114Ser + Asn140Ser + Gly165Asp +  
Ser207Asp + Ser220Glu  
Asn 68Asp + Ser109Glu + Gly139Glu + Ala204Ser +  
Leu221His + Gly223Asn  
Thr111Gly + Val138Pro + Gly139Asp + Ser207Glu +  
Ser216Glu + Ser220Asp  
Asn106Asp + Asn170Ser + Ser192Glu + Thr195Asp +

Trp208His + Ser220Glu  
 Asn106Asp + Gly162Asp + Asn166Gln + Thr167Asp +  
 Pro214Asp + Ala219Thr  
 Val115Glu + Gly136Glu + Ser207Glu + Tyr218Gly +  
 Ala219Pro + Leu221Pro  
 Gly136Ser + Gly162Asp + Asn163Gln + Ala164His +  
 Ser216Asp + Ser222Glu  
 Asn 68Ser + Thr 73Glu + Asn166Glu + Asn170Gln +  
 Tyr171Gly + Leu221Asp  
 Thr113Glu + Val115Cys + Thr137Glu + Ser191Glu +  
 Thr217Ser + Thr224Glu  
 Gly 67Asp + Ser107Asp + Gly139Asp + Gly165Pro +  
 Thr167Ser + Thr217Glu  
 Thr 73Gly + Gly108Asp + Thr137Glu + Gly162Glu +  
 Tyr210Gln + Ser220Asp  
 Leu104Asp + Gly139Pro + Tyr171Asp + Ser191Asp +  
 Ser222Glu + Thr224Ser  
 Thr113Asp + Pro169Glu + Tyr210Gln + Pro214Ser +  
 Ser220Glu + Gly223Asp  
 Gly108Asp + Gly136Glu + Ser141Asp + Thr195Asp +  
 Thr215Ser + Thr217Gln  
 Ser107Asp + Asn163Glu + Pro169Gly + Ser207Asp +  
 Trp208Asn + Gly223Asn  
 Gly110Asp + Asn163Asp + Ser191Asp + Thr195Gln +  
 Tyr210Glu + Thr217Pro  
 Val103Glu + Gly108Asp + Ala203Gly + Trp208Met +  
 Ala219Asp + Thr224Glu  
 Thr 73Gln + Thr111Asp + Leu134Asp + Gly136Ser +  
 Asn163Gln + Ser207Asp

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## II. Cleaning Compositions

In another embodiment of the present invention, an effective amount of one or more of the enzyme variants are included in compositions useful for cleaning a variety of surfaces in need of proteinaceous stain removal. Such cleaning compositions include detergent compositions for cleaning hard surfaces, unlimited in form (e.g., liquid and granular); detergent compositions for cleaning fabrics, unlimited in form (e.g., granular, liquid and bar formulations); dishwashing compositions (unlimited in form); oral cleaning compositions, unlimited in form (e.g., dentifrice, toothpaste and mouthwash formulations); denture cleaning compositions, unlimited in form (e.g., liquid, tablet); and contact lens cleaning compositions, unlimited in form (e.g., liquid, tablet).

The cleaning compositions also comprise, in addition to the Thermitase variants described hereinbefore, one or more cleaning composition materials compatible with the protease enzyme. the term

"cleaning composition material", as used herein, means any liquid, solid or gaseous material selected for the particular type of cleaning composition desired and the form of the product (e.g., liquid, granule, bar, spray, stick, paste, gel), which materials are also compatible with the Thermitase variant used in the composition. the specific selection of cleaning composition materials are readily made by considering the surface material to be cleaned, the desired form of the composition for the cleaning condition during use (e.g., through the wash detergent use). The term "compatible", as used herein, means the cleaning composition materials do not reduce the proteolytic activity of the Thermitase variant to such an extent that the protease is not effective as desired during normal use situations. Specific cleaning composition materials are exemplified in detail hereinafter.

As used herein, "effective amount of enzyme variant" refers to the quantity of enzyme variant necessary to achieve the enzymatic activity necessary in the specific cleaning composition. Such effective amounts are readily ascertained by one of ordinary skill in the art and is based on many factors, such as the particular enzyme variant used, the cleaning application, the specific composition of the cleaning composition, and whether a liquid or dry (e.g., granular, bar) composition is required, and the like. Preferably the cleaning compositions comprise from about 0.0001% to about 10% of one or more enzyme variants of the present invention, more preferably from about 0.001% to about 1%, more preferably still from about 0.01% to about 0.1%. Several examples of various cleaning compositions wherein the enzyme variants may be employed are discussed in further detail below. All parts, percentages and ratios used herein are by weight unless otherwise specified.

As used herein, "non-fabric cleaning compositions" include hard surface cleaning compositions, dishwashing compositions, oral cleaning compositions, denture cleaning compositions and contact lens cleaning compositions.

A. Cleaning Compositions for Hard Surfaces, Dishes and Fabrics

The enzyme variants of the present invention can be used in a variety of detergent compositions where high sudsing and good insoluble substrate removal are desired. Thus the enzyme variants can be used with various conventional ingredients to provide fully-formulated hard-surface cleaners, dishwashing compositions, fabric laundering compositions and the like. Such compositions can be in the form of liquids, granules, bars and

the like. Such compositions can be formulated as modern "concentrated" detergents which contain as much as 30%-60% by weight of surfactants.

The cleaning compositions herein can optionally, and preferably, contain various anionic, nonionic, zwitterionic, etc., surfactants. Such surfactants are typically present at levels of from about 5% to about 35% of the compositions.

Nonlimiting examples of surfactants useful herein include the conventional C<sub>11</sub>-C<sub>18</sub> alkyl benzene sulfonates and primary and random alkyl sulfates, the C<sub>10</sub>-C<sub>18</sub> secondary (2,3) alkyl sulfates of the formulas  $\text{CH}_3(\text{CH}_2)_x(\text{CHOSO}_3^- \text{M}^+)\text{CH}_3$  and  $\text{CH}_3(\text{CH}_2)_y(\text{CHOSO}_3^- \text{M}^+)\text{CH}_2\text{CH}_3$  wherein x and (y+1) are integers of at least about 7, preferably at least about 9, and M is a water-solubilizing cation, especially sodium, the C<sub>10</sub>-C<sub>18</sub> alkyl alkoxy sulfates (especially EO 1-5 ethoxy sulfates), C<sub>10</sub>-C<sub>18</sub> alkyl alkoxy carboxylates (especially the EO 1-5 ethoxycarboxylates), the C<sub>10</sub>-C<sub>18</sub> alkyl polyglycosides, and their corresponding sulfated polyglycosides, C<sub>12</sub>-C<sub>18</sub> alpha-sulfonated fatty acid esters, C<sub>12</sub>-C<sub>18</sub> alkyl and alkyl phenol alkoxyates (especially ethoxylates and mixed ethoxy/propoxy), C<sub>12</sub>-C<sub>18</sub> betaines and sulfobetaines ("sultaines"), C<sub>10</sub>-C<sub>18</sub> amine oxides, and the like. The alkyl alkoxy sulfates (AES) and alkyl alkoxy carboxylates (AEC) are preferred herein. (Use of such surfactants in combination with the aforesaid amine oxide and/or betaine or sultaine surfactants is also preferred, depending on the desires of the formulator.) Other conventional useful surfactants are listed in standard texts. Particularly useful surfactants include the C<sub>10</sub>-C<sub>18</sub> N-methyl glucamides disclosed in US Patent 5, 194,639, Connor et al., issued March 16, 1993, incorporated herein by reference.

A wide variety of other ingredients useful in detergent cleaning compositions can be included in the compositions herein, including other active ingredients, carriers, hydrotropes, processing aids, dyes or pigments, solvents for liquid formulations, etc. If an additional increment of sudsing is desired, suds boosters such as the C<sub>10</sub>-C<sub>16</sub> alkylamidates can be incorporated into the compositions, typically at about 1% to about 10% levels. The C<sub>10</sub>-C<sub>14</sub> monoethanol and diethanol amides illustrate a typical class of such suds boosters. Use of such suds boosters with high sudsing adjunct surfactants such as the amine oxides, betaines and sultaines noted above is also advantageous. If desired, soluble magnesium salts such as MgCl<sub>2</sub>, MgSO<sub>4</sub>, and the like, can be added at levels of, typically, from about

0.1% to about 2%, to provide additionally sudsing.

The liquid detergent compositions herein can contain water and other solvents as carriers. Low molecular weight primary or secondary alcohols exemplified by methanol, ethanol, propanol, and isopropanol are suitable. Monohydric alcohols are preferred for solubilizing surfactants, but polyols such as those containing from about 2 to about 6 carbon atoms and from about 2 to about 6 hydroxy groups (e.g., 1,3-propanediol, ethylene glycol, glycerine, and 1,2-propanediol) can also be used. The compositions may contain from about 5% to about 90%, typically from about 10% to about 50% of such carriers.

The detergent compositions herein will preferably be formulated such that during use in aqueous cleaning operations, the wash water will have a pH between about 6.8 and about 11.0. Finished products thus are typically formulated at this range. Techniques for controlling pH at recommended usage levels include the use of buffers, alkalis, acids, etc., and are well known to those skilled in the art.

When formulating the hard surface cleaning compositions and fabric cleaning compositions of the present invention, the formulator may wish to employ various builders at levels from about 5% to about 50% by weight. Typical builders include the 1-10 micron zeolites, polycarboxylates such as citrate and oxydisuccinates, layered silicates, phosphates, and the like. Other conventional builders are listed in standard formularies.

Likewise, the formulator may wish to employ various additional enzymes, such as cellulases, lipases, amylases and proteases in such compositions, typically at levels of from about 0.001% to about 1% by weight. Various detergent and fabric care enzymes are well-known in the laundry detergent art.

Various bleaching compounds, such as the percarbonates, perborates and the like, can be used in such compositions, typically at levels from about 1% to about 15% by weight. If desired, such compositions can also contain bleach activators such as tetraacetyl ethylenediamine, nonanoyloxybenzene sulfonate, and the like, which are also known in the art. Usage levels typically range from about 1% to about 10% by weight.

Various soil release agents, especially of the anionic oligoester type, various chelating agents, especially the aminophosphonates and ethylenediaminedisuccinates, various clay soil removal agents, especially ethoxylated tetraethylene pentamine, various dispersing agents, especially

polyacrylates and polyasparatates, various brighteners, especially anionic brighteners, various suds suppressors, especially silicones and secondary alcohols, various fabric softeners, especially smectite clays, and the like can all be used in such compositions at levels ranging from about 1% to about 35% by weight. Standard formularies and published patents contain multiple, detailed descriptions of such conventional materials.

Enzyme stabilizers may also be used in the cleaning compositions. Such enzyme stabilizers include propylene glycol (preferably from about 1% to about 10%), sodium formate (preferably from about 0.1% to about 1%) and calcium formate (preferably from about 0.1% to about 1%).

1. Hard surface cleaning compositions

As used herein "hard surface cleaning composition" refers to liquid and granular detergent compositions for cleaning hard surfaces such as floors, walls, bathroom tile, and the like. Hard surface cleaning compositions of the present invention comprise an effective amount of one or more enzyme variants of the present invention, preferably from about 0.001% to about 10%, more preferably from about .01% to about 5%, more preferably still from about .05% to about 1% by weight of active enzyme of the composition. In addition to comprising one or more of the enzyme variants, such hard surface cleaning compositions typically comprise a surfactant and a water-soluble sequestering builder. In certain specialized products such as spray window cleaners, however, the surfactants are sometimes not used since they may produce a filmy/streaky residue on the glass surface.

The surfactant component, when present, may comprise as little as 0.1% of the compositions herein, but typically the compositions will contain from about 0.25% to about 10%, more preferably from about 1% to about 5% of surfactant.

Typically the compositions will contain from about 0.5% to about 50% of a detergency builder, preferably from about 1% to about 10%.

Preferably the pH should be in the range of about 8 to 12. Conventional pH adjustment agents such as sodium hydroxide, sodium carbonate or hydrochloric acid can be used if adjustment is necessary.

Solvents may be included in the compositions. Useful solvents include, but are not limited to, glycol ethers such as diethyleneglycol monohexyl ether, diethyleneglycol monobutyl ether, ethyleneglycol monobutyl ether, ethyleneglycol monohexyl ether, propyleneglycol

monobutyl ether, dipropyleneglycol monobutyl ether, and diols such as 2,2,4-trimethyl-1,3-pentanediol and 2-ethyl-1,3-hexanediol. When used, such solvents are typically present at levels of from about 0.5% to about 15%, preferably from about 3% to about 11%.

Additionally, highly volatile solvents such as isopropanol or ethanol can be used in the present compositions to facilitate faster evaporation of the composition from surfaces when the surface is not rinsed after "full strength" application of the composition to the surface. When used, volatile solvents are typically present at levels of from about 2% to about 12% in the compositions.

The hard surface cleaning composition embodiment of the present invention is illustrated by the following examples.

#### Examples 7-12

Liquid Hard Surface Cleaning Compositions						
Component	Example No.					
	7	8	9	10	11	12
Gln66Asn	0.05	0.50	0.02	0.03	0.10	0.03
Gly206Asn	—	—	—	—	0.20	0.02
Na <sub>2</sub> DIDA*						
EDTA**	—	—	2.90	2.90	—	—
Na Citrate	—	—	—	—	2.90	2.90
NaC <sub>12</sub> Alkyl-benzene sulfonate	1.95	—	1.95	—	1.95	—
NaC <sub>12</sub> Alkylsulfate	—	2.20	—	2.20	—	2.20
NaC <sub>12</sub> (ethoxy)*** sulfate	—	2.20	—	2.20	—	2.20
C <sub>12</sub> Dimethylamine oxide	—	0.50	—	0.50	—	0.50
Na Cumene sulfonate	1.30	—	1.30	—	1.30	—
Hexyl Carbitol***	6.30	6.30	6.30	6.30	6.30	6.30
Water****	balance to 100%					

\*Disodium N-diethyleneglycol-N,N-iminodiacetate

\*\*Na<sub>4</sub> ethylenediamine diacetic acid

\*\*\*Diethyleneglycol monohexyl ether

\*\*\*\*All formulas adjusted to pH 7

In Examples 7-10, the Thermitase variants recited in Tables 2-36, among others, are substituted for Gln66Asn, with substantially similar



results.

In Examples 11-12, any combination of the Thermitase variants recited in Tables 2-36, among others, are substituted for Gln66Asn and Gly206Asn, with substantially similar results.

#### Examples 13-18

#### Spray Compositions for Cleaning Hard Surfaces and Removing Household Mildew

Component	Example No.					
	13	14	15	16	17	18
Thr111Asn	0.50	0.05	0.60	0.30	0.20	0.30
Thr217Gly + Ser222Glu	-	-	-	-	0.30	0.10
Sodium octyl sulfate	2.00	2.00	2.00	2.00	2.00	2.00
Sodium dodecyl sulfate	4.00	4.00	4.00	4.00	4.00	4.00
Sodium hydroxide	0.80	0.80	0.80	0.80	0.80	0.80
Silicate (Na)	0.04	0.04	0.04	0.04	0.04	0.04
Perfume	0.35	0.35	0.35	0.35	0.35	0.35
Water	balance to 100%					

Product pH is about 7.

In Examples 13-16, the Thermitase variants recited in Tables 2-36, among others, are substituted for Thr111Asn, with substantially similar results.

In Examples 17-18, any combination of the Thermitase variants recited in Tables 2-36, among others, are substituted for Thr111Asn and Thr217Gly + Ser222Glu, with substantially similar results.

#### 2. Dishwashing Compositions

In another embodiment of the present invention, dishwashing compositions comprise one or more enzyme variants of the present invention. As used herein, "dishwashing composition" refers to all forms for compositions for cleaning dishes, including but not limited to, granular and liquid forms. The dishwashing composition embodiment of the present invention is illustrated by the following examples.

Examples 19-24  
Dishwashing Composition

Component	Example No.					
	19	20	21	22	23	24
Gly139Asn	0.05	0.50	0.02	0.40	0.10	0.03
Ser207Glu + Tyr210Ser + Gly223Asn	-	-	-	-	0.40	0.02
C <sub>12</sub> -C <sub>14</sub> N-methyl- glucamide	0.90	0.90	0.90	0.90	0.90	0.90
C <sub>12</sub> ethoxy (1) sulfate	12.00	12.00	12.00	12.00	12.00	12.00
2-methyl undecanoic acid	4.50	4.50	4.50	4.50	4.50	4.50
C <sub>12</sub> ethoxy (2) carboxylate	4.50	4.50	4.50	4.50	4.50	4.50
C <sub>12</sub> alcohol ethoxylate (4)	3.00	3.00	3.00	3.00	3.00	3.00
C <sub>12</sub> amine oxide	3.00	3.00	3.00	3.00	3.00	3.00
Sodium cumene sulfonate	2.00	2.00	2.00	2.00	2.00	2.00
Ethanol	4.00	4.00	4.00	4.00	4.00	4.00
Mg <sup>++</sup> (as MgCl <sub>2</sub> )	0.20	0.20	0.20	0.20	0.20	0.20
Ca <sup>++</sup> (as CaCl <sub>2</sub> )	0.40	0.40	0.40	0.40	0.40	0.40
Water	balance to 100%					

Product pH is adjusted to 7.

In Examples 19-22, the Thermitase variants recited in Tables 2-36, among others, are substituted for Gly139Asn, with substantially similar results.

In Examples 23-24, any combination of the Thermitase variants recited in Tables 2-36, among others, are substituted for Gly139Asn and Ser207Glu + Tyr210Ser + Gly223Asn, with substantially similar results.

### 3. Fabric cleaning compositions

In another embodiment of the present invention, fabric cleaning compositions comprise one or more enzyme variants of the present invention. As used herein, "fabric cleaning composition" refers to all forms for detergent compositions for cleaning fabrics, including but not limited to, granular, liquid and bar forms. Preferred fabric cleaning compositions are those in the liquid form.

#### a. Granular fabric cleaning compositions

The granular fabric cleaning compositions of the present invention contain an effective amount of one or more enzyme variants of the present invention, preferably from about 0.001% to about 10%, more preferably from

about 0.005% to about 5%, more preferably from about 0.01% to about 1% by weight of active enzyme of the composition. In addition to one or more enzyme variants, the granular fabric cleaning compositions typically comprise at least one surfactant, one or more builders, and, in some cases, a bleaching agent.

The granular fabric cleaning composition embodiment of the present invention is illustrated by the following examples.

#### Examples 25-28

##### Granular Fabric Cleaning Composition

Component	Example No.			
	25	26	27	28
Ala168Asn	0.10	0.20	0.03	0.05
Tyr210Gly + Thr212Pro + Pro214Gly + Ser222Asp	-	-	0.02	0.05
C <sub>13</sub> linear alkyl benzene sulfonate	22.00	22.00	22.00	22.00
Phosphate (as sodium tripolyphosphates)	23.00	23.00	23.00	23.00
Sodium carbonate	23.00	23.00	23.00	23.00
Sodium silicate	14.00	14.00	14.00	14.00
Zeolite	8.20	8.20	8.20	8.20
Chelant (diethylenetriamine- pentaacetic acid)	0.40	0.40	0.40	0.40
Sodium sulfate	5.50	5.50	5.50	5.50
Water	balance to 100%			

In Examples 25-26, the Thermitase variants recited in Tables 2-36, among others, are substituted for Ala168Asn, with substantially similar results.

In Examples 27-28, any combination of the Thermitase variants recited in Tables 2-36, among others, are substituted for Ala168Asn and Tyr210Gly + Thr212Pro + Pro214Gly + Ser222Asp, with substantially similar results.

Examples 29-32  
Granular Fabric Cleaning Composition

Component	Example No.			
	29	30	31	32
Thr195Pro	0.10	0.20	0.03	0.05
Trp208Phe + Tyr213Met + Thr215Pro + Ser220Glu + Thr224Gly	-	-	0.02	0.05
C <sub>12</sub> alkyl benzene sulfonate	12.00	12.00	12.00	12.00
Zeolite A (1-10 micrometer)	26.00	26.00	26.00	26.00
2-butyl octanoic acid	4.00	4.00	4.00	4.00
C <sub>12</sub> -C <sub>14</sub> secondary (2,3) alkyl sulfate, Na salt	5.00	5.00	5.00	5.00
Sodium citrate	5.00	5.00	5.00	5.00
Optical brightener	0.10	0.10	0.10	0.10
Sodium sulfate	17.00	17.00	17.00	17.00
Water and minors	balance to 100%			

In Examples 29-30, the Thermitase variants recited in Tables 2-36, among others, are substituted for Thr195Pro, with substantially similar results.

In Examples 31-32, any combination of the Thermitase variants recited in Tables 2-36, among others, are substituted for Thr195Pro and Trp208Phe + Tyr213Met + Thr215Pro + Ser220Glu + Thr224Gly, with substantially similar results.

Examples 33-36  
Granular Fabric Cleaning Composition

Component	Example No.			
	33	34	35	36
Gly67Ser + Gly72Ser	0.10	0.20	0.03	0.05
Tyr171Thr	-	-	0.02	0.05
C <sub>13</sub> linear alkyl benzene sulfonate	22.00	22.00	22.00	22.00
Phosphate (as sodium tripolyphosphates)	23.00	23.00	23.00	23.00
Sodium carbonate	23.00	23.00	23.00	23.00
Sodium silicate	14.00	14.00	14.00	14.00
Zeolite	8.20	8.20	8.20	8.20
Chelant (diethylenetriamine-pentaacetic acid)	0.40	0.40	0.40	0.40
Sodium sulfate	5.50	5.50	5.50	5.50
Water	balance to 100%			

In Examples 33-34, the Thermitase variants recited in Tables 2-36, among others, are substituted for Gly67Ser + Gly72Ser, with substantially similar results.

In Examples 35-36, any combination of the Thermitase variants recited in Tables 2-36, among others, are substituted for Gly67Ser + Gly72Ser and Tyr171Thr, with substantially similar results.

Examples 37-40  
Granular Fabric Cleaning Composition

Component	Example No.			
	37	38	39	40
Ser192Asp + Phe193Leu + Thr195Asn	0.10	0.20	0.03	0.05
Tyr213Ser + Thr217Gly + Gly223Glu	-	-	0.02	0.05
C <sub>12</sub> alkyl benzene sulfonate	12.00	12.00	12.00	12.00
Zeolite A (1-10 micrometer)	26.00	26.00	26.00	26.00
2-butyl octanoic acid	4.00	4.00	4.00	4.00
C <sub>12</sub> -C <sub>14</sub> secondary (2,3) alkyl sulfate, Na salt	5.00	5.00	5.00	5.00
Sodium citrate	5.00	5.00	5.00	5.00
Optical brightener	0.10	0.10	0.10	0.10
Sodium sulfate	17.00	17.00	17.00	17.00
Water and minors	balance to 100%			

In Examples 37-38, the Thermitase variants recited in Tables 2-36, among others, are substituted for Ser192Asp + Phe193Leu + Thr195Asn, with substantially similar results.

In Examples 39-40, any combination of the Thermitase variants recited in Tables 2-36, among others, are substituted for Ser192Asp + Phe193Leu + Thr195Asn and Tyr213Ser + Thr217Gly + Gly223Glu, with substantially similar results.

Examples 41-42  
Granular Fabric Cleaning Composition

Component	Example No.	
	41	42
Linear alkyl benzene sulphonate	11.4	10.70
Tallow alkyl sulphate	1.80	2.40
C <sub>14-15</sub> alkyl sulphate	3.00	3.10
C <sub>14-15</sub> alcohol 7 times ethoxylated	4.00	4.00
Tallow alcohol 11 times ethoxylated	1.80	1.80
Dispersant	0.07	0.1
Silicone fluid	0.80	0.80
Trisodium citrate	14.00	15.00
Citric acid	3.00	2.50
Zeolite	32.50	32.10
Maleic acid acrylic acid copolymer	5.00	5.00
Diethylene triamine penta methylene phosphonic acid	1.00	0.20
Leu104Asp + Gly139Pro + Tyr171Asp + Ser191Asp + Ser222Glu + Thr224Ser	0.30	0.30
Lipase	0.36	0.40
Amylase	0.30	0.30
Sodium silicate	2.00	2.50
Sodium sulphate	3.50	5.20
Polyvinyl pyrrolidone	0.30	0.50
Perborate	0.5	1
Phenol sulphonate	0.1	0.2
Peroxidase	0.1	0.1
Minors	Up to 100	Up to 100

Examples 43-44  
Granular Fabric Cleaning Composition

Component	Example No.	
	43	44
Sodium linear C <sub>12</sub> alkyl benzene-sulfonate	6.5	8.0
Sodium sulfate	15.0	18.0
Zeolite A	26.0	22.0
Sodium nitrilotriacetate	5.0	5.0
Polyvinyl pyrrolidone	0.5	0.7
Tetraacetylene diamine	3.0	3.0
Boric acid	4.0	-
Perborate	0.5	1
Phenol sulphonate	0.1	0.2
Pro205Gly + Trp208Leu + Thr217Gln + Leu221Gln + Gly223Gln + Thr224Glu	0.4	0.4
Fillers (e.g., silicates; carbonates; perfumes; water)	Up to 100	Up to 100

Example 45  
Compact Granular Fabric Cleaning Composition

Component	Weight %
Alkyl Sulphate	8.0
Alkyl Ethoxy Sulphate	2.0
Mixture of C <sub>25</sub> and C <sub>45</sub> alcohol 3 and 7 times ethoxylated	6.0
Polyhydroxy fatty acid amide	2.5
Zeolite	17.0
Layered silicate/citrate	16.0
Carbonate	7.0
Maleic acid acrylic acid copolymer	5.0
Soil release polymer	0.4
Carboxymethyl cellulose	0.4
Poly (4-vinylpyridine) -N-oxide	0.1
Copolymer of vinylimidazole and vinylpyrrolidone	0.1
PEG2000	0.2
Asn66Ser + Ser70Glu + Gly72Asn	0.5
Lipase	0.2
Cellulase	0.2
Tetracetylene diamine	6.0

Percarbonate	22.0
Ethylene diamine disuccinic acid	0.3
Suds suppressor	3.5
Disodium-4,4'-bis (2-morpholino -4-anilino-s-triazin-6-ylamino) stilbene-2,2'-disulphonate	0.25
Disodium-4,4'-bis (2-sulfostyryl) biphenyl	0.05
Water, Perfume and Minors	Up to 100

## Example 46

## Granular Fabric Cleaning Composition

Component	Weight %
Linear alkyl benzene sulphonate	7.6
C <sub>16</sub> -C <sub>18</sub> alkyl sulfate	1.3
C <sub>14</sub> -15 alcohol 7 times ethoxylated	4.0
Coco-alkyl-dimethyl hydroxyethyl ammonium chloride	1.4
Dispersant	0.07
Silicone fluid	0.8
Trisodium citrate	5.0
Zeolite 4A	15.0
Maleic acid acrylic acid copolymer	4.0
Diethylene triamine penta methylene phosphonic acid	0.4
Perborate	15.0
Tetraacetylene diamine	5.0
Smectite clay	10.0
Poly (oxy ethylene) (MW 300,000)	0.3
Ser109Glu + Thr113Gly	0.4
Lipase	0.2
Amylase	0.3
Cellulase	0.2
Sodium silicate	3.0
Sodium carbonate	10.0
Carboxymethyl cellulose	0.2
Brighteners	0.2
Water, perfume and minors	Up to 100



## Example 47

## Granular Fabric Cleaning Composition

Component	Weight %
Linear alkyl benzene sulfonate	6.92
Tallow alkyl sulfate	2.05
C <sub>14-15</sub> alcohol 7 times ethoxylated	4.4
C <sub>12-15</sub> alkyl ethoxy sulfate - 3 times ethoxylated	0.16
Zeolite	20.2
Citrate	5.5
Carbonate	15.4
Silicate	3.0
Maleic acid acrylic acid copolymer	4.0
Carboxymethyl cellulase	0.31
Soil release polymer	0.30
Gly135Gln + Val138Asp + Gly139Ser	0.2
Lipase	0.36
Cellulase	0.13
Perborate tetrahydrate	11.64
Perborate monohydrate	8.7
Tetraacetylene diamine	5.0
Diethylene tramine penta methyl phosphonic acid	0.38
Magnesium sulfate	0.40
Brightener	0.19
Perfume, silicone, suds suppressors	0.85
Minors	Up to 100

b. Liquid fabric cleaning compositions

- Liquid fabric cleaning compositions of the present invention comprise an effective amount of one or more enzyme variants of the present invention, preferably from about 0.005% to about 5%, more preferably from about 0.01% to about 1%, by weight of active enzyme of the composition. Such liquid fabric cleaning compositions typically additionally comprise an anionic surfactant, a fatty acid, a water-soluble detergency builder and water.

The liquid fabric cleaning composition embodiment of the present invention is illustrated by the following examples.

## Examples 48-52

## Liquid Fabric Cleaning Compositions

Component	Example No.				
	48	49	50	51	52
Asn166Asp + Thr167Asn + Asn170Ser + Tyr171Thr	0.05	0.03	0.30	0.03	0.10
Ser191Glu + Ser192Asp + Phe193Asp + Thr195Pro	-	-	-	0.01	0.20
C <sub>12</sub> - C <sub>14</sub> alkyl sulfate, Na	20.00	20.00	20.00	20.00	20.00
2-butyl octanoic acid	5.00	5.00	5.00	5.00	5.00
Sodium citrate	1.00	1.00	1.00	1.00	1.00
C <sub>10</sub> alcohol ethoxylate (3)	13.00	13.00	13.00	13.00	13.00
Monethanolamine	2.50	2.50	2.50	2.50	2.50
Water/propylene glycol/ethanol (100:1:1)	balance to 100%				

In Examples 48-50 the Thermitase variants recited in Tables 2-36, among others, are substituted for Asn166Asp + Thr167Asn + Asn170Ser + Tyr171Thr, with substantially similar results.

In Examples 51-52, any combination of the Thermitase variants recited in Tables 2-36, among others, are substituted for Asn166Asp + Thr167Asn + Asn170Ser + Tyr171Thr and Ser191Glu + Ser192Asp + Phe193Asp + Thr195Pro, with substantially similar results.

## Examples 53-57

## Liquid Fabric Cleaning Compositions

Component	Example No.				
	53	54	55	56	57
Pro205Gln + Gly206Ser + Trp208Val + Tyr210Leu + Thr212Asn + Tyr218Met + Ser220Glu	0.05	0.03	0.30	0.03	0.10
Asn 68Asp + Val138Asp + Ala164Gly + Ala168Glu + Thr217Gly	-	-	-	0.01	0.20
C <sub>12</sub> - C <sub>14</sub> alkyl sulfate, Na	20.00	20.00	20.00	20.00	20.00
2-butyl octanoic acid	5.00	5.00	5.00	5.00	5.00
Sodium citrate	1.00	1.00	1.00	1.00	1.00
C <sub>10</sub> alcohol ethoxylate (3)	13.00	13.00	13.00	13.00	13.00
Monethanolamine	2.50	2.50	2.50	2.50	2.50
Water/propylene glycol/ethanol (100:1:1)	balance to 100%				

In Examples 53-55 the Thermitase variants recited in Tables 2-36, among others, are substituted for Pro205Gln + Gly206Ser + Trp208Val + Tyr210Leu + Thr212Asn + Tyr218Met + Ser220Glu, with substantially similar results.

In Examples 56-57, any combination of the Thermitase variants recited in Tables 2-36, among others, are substituted for Pro205Gln + Gly206Ser + Trp208Val + Tyr210Leu + Thr212Asn + Tyr218Met + Ser220Glu and Asn 68Asp + Val138Asp + Ala164Gly + Ala168Glu + Thr217Gly, with substantially similar results.

#### Examples 58-59

##### Liquid Fabric Cleaning Composition

Component	Example No.	
	58	59
C <sub>12-14</sub> alkenyl succinic acid	3.0	8.0
Citric acid monohydrate	10.0	15.0
Sodium C <sub>12-15</sub> alkyl sulphate	8.0	8.0
Sodium sulfate of C <sub>12-15</sub> alcohol 2 times ethoxylated	-	3.0
C <sub>12-15</sub> alcohol 7 times ethoxylated	-	8.0
C <sub>12-15</sub> alcohol 5 times ethoxylated	8.0	-
Diethylene triamine penta (methylene phosphonic acid)0.2	-	-
Oleic acid	1.8	-
Ethanol	4.0	4.0
Propanediol	2.0	2.0
Leu134Cys + Thr137Glu + Val138Gly	0.2	0.2
Polyvinyl pyrrolidone	1.0	2.0
Suds suppressor	0.15	0.15
NaOH	up to pH 7.5	
Perborate	0.5	1
Phenol sulphonate	0.1	0.2
Peroxidase	0.4	0.1
Waters and minors	up to 100 parts	

In each of Examples 58 and 59 herein, the Thermitase variants recited in Tables 2-36, among others, are substituted for Leu134Cys + Thr137Glu + Val138Gly, with substantially similar results.

## Examples 60-62

## Liquid Fabric Cleaning Composition

Component	Example No.		
	60	61	62
Citric Acid	7.10	3.00	3.00
Fatty Acid	2.00	-	2.00
Ethanol	1.93	3.20	3.20
Boric Acid	2.22	3.50	3.50
Monoethanolamine	0.71	1.09	1.09
1,2 Propanediol	7.89	8.00	8.00
NaCumene Sulfonate	1.80	3.00	3.00
NaFormate	0.08	0.08	0.08
NaOH	6.70	3.80	3.80
Silicon anti-foam agent	1.16	1.18	1.18
Gly67Glu + Ser70Asp + Gly72Ser + Thr73Ser	0.0145	-	-
Ile209Ala + Ala219Pro	-	0.0145	-
Leu216Asn	-	-	0.0145
Lipase	0.200	0.200	0.200
Cellulase	-	7.50	7.50
Soil release polymer	0.29	0.15	0.15
Anti-foaming agents	0.06	0.085	0.085
Brightener 36	0.095	-	-
Brightener 3	-	0.05	0.05
C <sub>12</sub> alkyl benzenesulfonic acid	9.86	-	-
C <sub>12-15</sub> alkyl polyethoxylate (2.5) sulfate	13.80	18.00	18.00
C <sub>12</sub> glucose amide	-	5.00	5.00
C <sub>12-13</sub> alkyl polyethoxylate (9)	2.00	2.00	2.00
Water, perfume and minors	balance to 100%		

c. Bar fabric cleaning compositions

Bar fabric cleaning compositions of the present invention suitable for hand-washing soiled fabrics contain an effective amount of one or more enzyme variants of the present invention, preferably from about 0.001% to about 10%, more preferably from about 0.01% to about 1% by weight of the composition.

The bar fabric cleaning composition embodiment of the present invention is illustrated by the following examples.

Examples 63-66  
Bar Fabric Cleaning Compositions

Component	Example No.			
	63	64	65	66
Trp112Cys + Thr217Gly	0.3	-	0.1	0.02
Val103Ala + Thr212Ser	-	-	0.4	0.03
C <sub>12</sub> -C <sub>16</sub> alkyl sulfate, Na	20.0	20.0	20.0	20.00
C <sub>12</sub> -C <sub>14</sub> N-methyl glucamide	5.0	5.0	5.0	5.00
C <sub>11</sub> -C <sub>13</sub> alkyl benzene sulfonate, Na	10.0	10.0	10.0	10.00
Sodium carbonate	25.0	25.0	25.0	25.00
Sodium pyrophosphate	7.0	7.0	7.0	7.00
Sodium tripolyphosphate	7.0	7.0	7.0	7.00
Zeolite A (0.1-.10 $\mu$ )	5.0	5.0	5.0	5.00
Carboxymethylcellulose	0.2	0.2	0.2	0.20
Polyacrylate (MW 1400)	0.2	0.2	0.2	0.20
Coconut monethanolamide	5.0	5.0	5.0	5.00
Brightener, perfume	0.2	0.2	0.2	0.20
CaSO <sub>4</sub>	1.0	1.0	1.0	1.00
MgSO <sub>4</sub>	1.0	1.0	1.0	1.00
Water	4.0	4.0	4.0	4.00
Filler*	balance to 100%			

\*Can be selected from convenient materials such as CaCO<sub>3</sub>, talc, clay, silicates, and the like.

In Examples 63-64 the Thermitase variants recited in Tables 2-36, among others, are substituted for Trp112Cys + Thr217Gly, with substantially similar results.

- In Examples 65-66, any combination of the Thermitase variants recited in Tables 2-36, among others, are substituted for Trp112Cys + Thr217Gly and Val103Ala + Thr212Ser, with substantially similar results.

Examples 67-70  
Bar Fabric Cleaning Compositions

Component	Example No.			
	67	68	69	70
Ser109Glu	0.3	-	0.1	0.02
Pro169Glu	-	0.3	0.4	0.03
C <sub>12</sub> -C <sub>16</sub> alkyl sulfate, Na	20.0	20.0	20.0	20.00
C <sub>12</sub> -C <sub>14</sub> N-methyl glucamide	5.0	5.0	5.0	5.00
C <sub>11</sub> -C <sub>13</sub> alkyl benzene sulfonate, Na	10.0	10.0	10.0	10.00
Sodium carbonate	25.0	25.0	25.0	25.00
Sodium pyrophosphate	7.0	7.0	7.0	7.00
Sodium tripolyphosphate	7.0	7.0	7.0	7.00
Zeolite A (0.1-10 $\mu$ )	5.0	5.0	5.0	5.00
Carboxymethylcellulose	0.2	0.2	0.2	0.20
Polyacrylate (MW 1400)	0.2	0.2	0.2	0.20
Coconut monethanolamide	5.0	5.0	5.0	5.00
Brightener, perfume	0.2	0.2	0.2	0.20
CaSO <sub>4</sub>	1.0	1.0	1.0	1.00
MgSO <sub>4</sub>	1.0	1.0	1.0	1.00
Water	4.0	4.0	4.0	4.00
Filler*	balance to 100%			

\*Can be selected from convenient materials such as CaCO<sub>3</sub>, talc, clay, silicates, and the like.

In Example 67, the Thermitase variants recited in Tables 2-36, among others, are substituted for Ser109Glu, with substantially similar results.

In Example 68, the Thermitase variants recited in Tables 2-36, among others, are substituted for Pro169Glu, with substantially similar results.

In Examples 69-70, any combination of the Thermitase variants recited in Tables 2-36, among others, are substituted for Ser109Glu and Pro169Glu, with substantially similar results.

**B. Additional Cleaning Compositions**

In addition to the hard surface cleaning, dishwashing and fabric cleaning compositions discussed above, one or more enzyme variants of the present invention may be incorporated into a variety of other cleaning compositions where hydrolysis of an insoluble substrate is desired. Such

additional cleaning compositions include but are not limited to, oral cleaning compositions, denture cleaning compositions, and contact lens cleaning compositions.

1. Oral cleaning compositions

In another embodiment of the present invention, a pharmaceutically-acceptable amount of one or more enzyme variants of the present invention are included in compositions useful for removing proteinaceous stains from teeth or dentures. As used herein, "oral cleaning compositions" refers to dentifrices, toothpastes, toothgels, toothpowders, mouthwashes, mouth sprays, mouth gels, chewing gums, lozenges, sachets, tablets, biogels, prophylaxis pastes, dental treatment solutions, and the like. Preferably, the oral cleaning compositions comprise from about 0.0001% to about 20% of one or more enzyme variants of the present invention, more preferably from about 0.001% to about 10%, more preferably still from about 0.01% to about 5%, by weight of the composition, and a pharmaceutically-acceptable carrier. As used herein, "pharmaceutically-acceptable" means that drugs, medicaments or inert ingredients which the term describes are suitable for use in contact with the tissues of humans and lower animals without undue toxicity, incompatibility, instability, irritation, allergic response, and the like, commensurate with a reasonable benefit/risk ratio.

Typically, the pharmaceutically-acceptable oral cleaning carrier components of the oral cleaning compositions will generally comprise from about 50% to about 99.99%, preferably from about 65% to about 99.99%, more preferably from about 65% to about 99%, by weight of the composition.

The pharmaceutically-acceptable carrier components and optional components which may be included in the oral cleaning compositions of the present invention are well known to those skilled in the art. A wide variety of composition types, carrier components and optional components useful in the oral cleaning compositions are disclosed in U.S. Patent 5,096,700, Seibel, issued March 17, 1992; U.S. Patent 5,028,414, Sampathkumar, issued July 2, 1991; and U.S. Patent 5,028,415, Benedict, Bush and Sunberg, issued July 2, 1991; all of which are incorporated herein by reference.

The oral cleaning composition embodiment of the present invention is illustrated by the following examples.

Examples 71-74  
Dentifrice Composition

Component	Example No.			
	71	72	73	74
Tyr218Ala	2.000	3.500	1.500	2.000
Sorbitol (70% aqueous solution)	35.000	35.000	35.000	35.000
PEG-6*	1.000	1.000	1.000	1.000
Silica dental abrasive**	20.000	20.000	20.000	20.000
Sodium fluoride	0.243	0.243	0.243	0.243
Titanium dioxide	0.500	0.500	0.500	0.500
Sodium saccharin	0.286	0.286	0.286	0.286
Sodium alkyl sulfate (27.9% aqueous solution)	4.000	4.000	4.000	4.000
Flavor	1.040	1.040	1.040	1.040
Carboxyvinyl Polymer***	0.300	0.300	0.300	0.300
Carrageenan****	0.800	0.800	0.800	0.800
Water	balance to 100%			

\*PEG-6 = Polyethylene glycol having a molecular weight of 600.

\*\*Precipitated silica identified as Zeodent 119 offered by J.M. Huber.

\*\*\*Carbopol offered by B.F. Goodrich Chemical Company.

\*\*\*\*Iota Carrageenan offered by Hercules Chemical Company.

In Examples 71-74 the Thermitase variants recited in Tables 2-36, among others, are substituted for Tyr218Ala, with substantially similar results.



Examples 75-78  
Mouthwash Composition

Component	Example No.			
	75	76	77	78
Ala164His + Ser220Glu	3.00	7.50	1.00	5.00
SDA 40 Alcohol	8.00	8.00	8.00	8.00
Flavor	0.08	0.08	0.08	0.08
Emulsifier	0.08	0.08	0.08	0.08
Sodium Fluoride	0.05	0.05	0.05	0.05
Glycerin	10.00	10.00	10.00	10.00
Sweetener	0.02	0.02	0.02	0.02
Benzoic acid	0.05	0.05	0.05	0.05
Sodium hydroxide	0.20	0.20	0.20	0.20
Dye	0.04	0.04	0.04	0.04
Water	balance to 100%			

In Examples 75-78, the Thermitase variants recited in Tables 2-36, among others, are substituted for Ala164His + Ser220Glu, with substantially similar results.

Examples 79-82  
Lozenge Composition

Component	Example No.			
	79	80	81	82
Leu221Val + Thr224Gln	0.01	0.03	0.10	0.02
Sorbitol	17.50	17.50	17.50	17.50
Mannitol	17.50	17.50	17.50	17.50
Starch	13.60	13.60	13.60	13.60
Sweetener	1.20	1.20	1.20	1.20
Flavor	11.70	11.70	11.70	11.70
Color	0.10	0.10	0.10	0.10
Corn Syrup	balance to 100%			

In Examples 79-82, the Thermitase variants recited in Tables 2-36, among others, are substituted for Ser Leu221Val + Thr224Gln, with substantially similar results.

Examples 83-86  
Chewing Gum Composition

Component	Example No.			
	83	84	85	86
Ser191Asp + Phe193Ile + Thr195Gly	0.03	0.02	0.10	0.05
Sorbitol crystals	38.44	38.40	38.40	38.40
Paloja-T gum base*	20.00	20.00	20.00	20.00
Sorbitol (70% aqueous solution)	22.00	22.00	22.00	22.00
Mannitol	10.00	10.00	10.00	10.00
Glycerine	7.56	7.56	7.56	7.56
Flavor	1.00	1.00	1.00	1.00

\*Supplied by L.A. Dreyfus Company.

In Examples 83-86, the Thermitase variants recited in Tables 2-36, among others, are substituted for Ser191Asp + Phe193Ile + Thr195Gly, with substantially similar results.

## 2. Denture cleaning compositions

In another embodiment of the present invention, denture cleaning compositions for cleaning dentures outside of the oral cavity comprise one or more enzyme variants of the present invention. Such denture cleaning compositions comprise an effective amount of one or more of the enzyme variants, preferably from about 0.0001% to about 50% of one or more of the enzyme variants, more preferably from about 0.001% to about 35%, more preferably still from about 0.01% to about 20%, by weight of the composition, and a denture cleansing carrier. Various denture cleansing composition formats such as effervescent tablets and the like are well known in the art (see for example U.S. Patent 5,055,305, Young, incorporated herein by reference), and are generally appropriate for incorporation of one or more of the enzyme variants for removing proteinaceous stains from dentures.

The denture cleaning composition embodiment of the present invention is illustrated by the following examples.

## Examples 87-90

## Two-layer Effervescent Denture Cleansing Tablet

Component	Example No.			
	87	88	89	90
<u>Acidic Layer</u>				
Gly135Gln + Gly139Asn + Asn140Gln + Ser141Asp	1.0	1.5	0.01	0.05
Tartaric acid	24.0	24.0	24.00	24.00
Sodium carbonate	4.0	4.0	4.00	4.00
Sulphamic acid	10.0	10.0	10.00	10.00
PEG 20,000	4.0	4.0	4.00	4.00
Sodium bicarbonate	24.5	24.5	24.50	24.50
Potassium persulfate	15.0	15.0	15.00	15.00
Sodium acid pyrophosphate	7.0	7.0	7.00	7.00
Pyrogenic silica	2.0	2.0	2.00	2.00
TAED*	7.0	7.0	7.00	7.00
Ricinoleylsulfosuccinate	0.5	0.5	0.50	0.50
Flavor	1.0	1.0	1.00	1.00
<u>Alkaline Layer</u>				
Sodium perborate monohydrate	32.0	32.0	32.00	32.00
Sodium bicarbonate	19.0	19.0	19.00	19.00
EDTA	3.0	3.0	3.00	3.00
Sodium tripolyphosphate	12.0	12.0	12.00	12.00
PEG 20,000	2.0	2.0	2.00	2.00
Potassium persulfate	26.0	26.0	26.00	26.00
Sodium carbonate	2.0	2.0	2.00	2.00
Pyrogenic silica	2.0	2.0	2.00	2.00
Dye/flavor	2.0	2.0	2.00	2.00

\*Tetraacetylene diamine

In Examples 87-90, the Thermitase variants recited in Tables 2-36, among others, are substituted for Gly135Gln + Gly139Asn + Asn140Gln + Ser141Asp, with substantially similar results.

### 3. Contact Lens Cleaning Compositions

In another embodiment of the present invention, contact lens cleaning compositions comprise one or more enzyme variants of the present invention. Such contact lens cleaning compositions comprise an effective amount of one or more of the enzyme variants, preferably from about 0.01%

to about 50% of one or more of the enzyme variants, more preferably from about 0.01% to about 20%, more preferably still from about 1% to about 5%, by weight of the composition, and a contact lens cleaning carrier. Various contact lens cleaning composition formats such as tablets, liquids and the like are well known in the art (see for example U.S. Patent 4,863,627, Davies, Meaken and Rees, issued September 5, 1989; U.S. Patent Re. 32,672, Huth, Lam and Kirai, reissued May 24, 1988; U.S. Patent 4,609,493, Schäfer, issued September 2, 1986; U.S. Patent, 4,690,793, Ogunbiyi and Smith, issued September 1, 1987; U.S. Patent 4,614,549, Ogunbiyi, Riedhammer and Smith, issued September 30, 1986; and U.S. Patent 4,285,738, Ogata, issued August 25, 1981; each of which are incorporated herein by reference), and are generally appropriate for incorporation of one or more enzyme variants of the present invention for removing proteinaceous stains from contact lens.

The contact lens cleaning composition embodiment of the present invention is illustrated by the following examples.

#### Examples 91-94

##### Enzymatic Contact Lens Cleaning Solution

Component	Example No.			
	91	92	93	94
Leu221Gln	0.01	0.5	0.1	2.0
Glucose	50.00	50.0	50.0	50.0
Nonionic surfactant (polyoxyethylene-polyoxypropylene copolymer)	2.00	2.0	2.0	2.0
Anionic surfactant (polyoxyethylene-alkylphenylether sodium sulfricester)	1.00	1.0	1.0	1.0
Sodium chloride	1.00	1.0	1.0	1.0
Borax	0.30	0.3	0.3	0.3
Water	balance to 100%			

In Examples 91-94, the Thermitase variants recited in Tables 2-36, among others, are substituted for Leu221Gln, with substantially similar results.

While particular embodiments of the subject invention have been described, it will be obvious to those skilled in the art that various changes and modifications of the subject invention can be made without departing

from the spirit and scope of the invention. It is intended to cover, in the appended claims, all such modifications that are within the scope of the invention.

(i) APPLICANT: BRODE, PHILIP F.  
BARNETT, BOBBY L.  
RUBINGH, DONN N.

(iii) NUMBER OF SEQUENCES: 1

(A) ADDRESSEE: THE PROCTER & GAMBLE COMPANY  
(B) STREET: 11810 East Miami River Road  
(C) CITY: Ross  
(D) STATE: OH  
(E) COUNTRY: USA  
(F) ZIP: 45061

(A) MEDIUM TYPE: Floppy disk  
(B) COMPUTER: IBM PC compatible  
(C) OPERATING SYSTEM: PC-DOS/MS-DOS  
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30

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(A) NAME: CORSTANJE, BRAHM J.  
(B) REGISTRATION NUMBER: 34,804  
(C) REFERENCE/DOCKET NUMBER: 5607

(A) TELEPHONE: (513) 627-2858  
(B) TELEFAX: (513) 627-0260

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 279 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

Gln	Tyr	Thr	Pro	Asn	Asp	Pro	Tyr	Phe	Ser	Ser	Arg	Gln	Tyr	Gly	Pro
	1				5				10						15
Ala	Lys	Ile	Gln	Ala	Pro	Gln	Ala	Trp	Asp	Ile	Ala	Glu	Gly	Ser	Gly
				20					25					30	
Leu	Lys	Ile	Ala	Ile	Val	Asp	Thr	Gly	Val	Gln	Ser	Asn	His	Pro	Asp
			35					40					45		
Thr	Ala	Gly	Lys	Val	Val	Gly	Gly	Trp	Asp	Phe	Val	Asp	Asn	Asp	Ser

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	50	55	60	
Ala	Pro	Gln	Asn	Gly
	Asn	Gly	His	Gly
	Thr	His	Cys	Ala
	Gly	Ile	Ala	
65		70	75	80
Ala	Ala	Val	Thr	Asn
	Asn	Ser	Thr	Gly
	Ile	Ala	Gly	Thr
	Ala	Pro	Lys	
	85	90	95	
Trp	Ser	Ile	Leu	Ala
	Val	Arg	Val	Leu
	Asp	Asn	Ser	Gly
	Ser	Gly	Ser	Gly
	Thr			
	100	105	110	
Lys	Thr	Ala	Val	Ala
	Asn	Gly	Ile	Thr
	Tyr	Ala	Ala	Asp
	Gln	Gly	Ala	
	115	120	125	
Gln	Val	Ile	Ser	Leu
	Ser	Leu	Gly	Gly
	Thr	Val	Gly	Asn
	Ser	Gly	Leu	
	130	135	140	
Ala	Gln	Ala	Val	Asn
	Tyr	Ala	Trp	Asn
	Lys	Gly	Ser	Val
	Val	Val	Ala	
145	150	155		
160				
Ser	Ala	Gly	Asn	Ala
	Gly	Asn	Thr	Ala
	Pro	Asn	Tyr	Pro
	Ala	Tyr	Tyr	
	165	170	175	
Ser	Asn	Ala	Ile	Ala
	Val	Ala	Ser	Thr
	Asp	Gln	Asn	Asp
	Asn	Lys	Ser	
	180	185	190	
Trp	Phe	Ser	Thr	Tyr
	Gly	Ser	Val	Val
	Asp	Val	Ala	Ala
	Pro	Gly	Ser	
	195	200	205	
Thr	Ile	Tyr	Ser	Thr
	Tyr	Pro	Thr	Ser
	Thr	Tyr	Ala	Ser
	Leu	Ser	Gly	
	210	215	220	
Ser	Ser	Met	Ala	Thr
	Pro	His	Val	Ala
	Gly	Val	Ala	Gly
	Leu	Leu	Ala	
225	230	235		
240				
Ala	Gln	Gly	Arg	Ser
	Ala	Ser	Asn	Ile
	Arg	Ala	Ala	Ile
	Glu	Asn	Thr	
	245	250	255	
Asn	Asp	Lys	Ile	Ser
	Gly	Thr	Gly	Thr
	Tyr	Trp	Ala	Lys
	Gly	Arg	Val	
	260	265	270	
	Ala	Tyr	Lys	Ala
	Val	Gln	Tyr	
	275			

What is Claimed is:

1. A Thermitase variant having a modified amino acid sequence of Thermitase wild-type amino acid sequence, the wild-type amino acid sequence comprising a first loop region, a second loop region, a third loop region, a fourth loop region, a fifth loop region and a sixth loop region; wherein the modified amino acid sequence comprises a substitution at one or more positions in one or more of the loop regions; wherein

- A. when a substitution occurs in the first loop region, the substitution occurs at one or more of positions 66, 69, 70, 72 or 73; wherein
  - a. when a substitution occurs at position 66, the substituting amino acid is Asn;
  - b. when a substitution occurs at position 69, the substituting amino acid is Gln;
  - c. when a substitution occurs at position 70, the substituting amino acid is Asn, Asp, Gln, Glu, Pro or Ser;
  - d. when a substitution occurs at position 72, the substituting amino acid is Asn, Asp, Gln, Glu, Pro or Ser; and
  - e. when a substitution occurs at position 73, the substituting amino acid is Asn, Asp, Gln, Glu, Pro or Ser;
- B. when a substitution occurs in the second loop region, the substitution occurs at one or more of positions 103, 104, 105, 106, 108, 110, 112, 113 or 114; wherein
  - a. when a substitution occurs at position 103, the substituting amino acid is Ala, Asn, Asp, Cys, Gln, Glu, Gly, His, Met, Pro, Ser or Thr;
  - b. when a substitution occurs at position 104, the substituting amino acid is Ala, Asn, Asp, Cys, Gln, Gly, His, Ile, Met, Pro or Ser;
  - c. when a substitution occurs at position 105, the substituting amino acid is Glu;
  - d. when a substitution occurs at position 106, the substituting amino acid is Gln;



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- e. when a substitution occurs at position 108, the substituting amino acid is Asn, Gln, Pro or Ser;
  - f. when a substitution occurs at position 110, the substituting amino acid is Asn, Asp, Gln, Glu, Pro or Ser;
  - g. when a substitution occurs at position 112, the substituting amino acid is Asn, Asp, Cys, Gln, Glu, His, Ile, Met, Phe, Pro, Thr or Tyr;
  - h. when a substitution occurs at position 113, the substituting amino acid is Asn, Asp, Gln, Glu, Pro or Ser; and
  - i. when a substitution occurs at position 114, the substituting amino acid is Asn, Asp, Gln, Glu, Gly, His, Pro, Ser or Thr;
- C. when a substitution occurs in the third loop region, the substitution occurs at one or more of positions 134, 135, 136, 137, 138, 139 or 141; wherein
- a. when a substitution occurs at position 134, the substituting amino acid is Ala, Asn, Cys, Gln, Gly, His, Met, Pro, Ser, Thr or Val;
  - b. when a substitution occurs at position 135, the substituting amino acid is Asn, Asp, Gln, Glu, Pro or Ser;
  - c. when a substitution occurs at position 136, the substituting amino acid is Pro;
  - d. when a substitution occurs at position 137, the substituting amino acid is Asn, Asp, Gln, Glu or Ser;
  - e. when a substitution occurs at position 138, the substituting amino acid is Ala, Asn, Asp, Cys, Gln, Glu, Gly, His, Met, Pro, Ser or Thr;
  - f. when a substitution occurs at position 139, the substituting amino acid is Asn, Gln, Pro or Ser; and
  - g. when a substitution occurs at position 141, the substituting amino acid is Asp or Glu;
- D. when a substitution occurs in the fourth loop region, the substitution occurs at one or more of positions 167, 169, or 171; wherein

- a. when a substitution occurs at position 167, the substituting amino acid is Asn, Asp, Gln, Glu, Pro or Ser;
- b. when a substitution occurs at position 169, the substituting amino acid is Asn, Asp, Gln, Glu, Gly or Ser; and
- c. when a substitution occurs at position 171, the substituting amino acid is His, Ile, Leu, Met or Pro;
- E. when a substitution occurs in the fifth loop region, the substitution occurs at one or more of position 193; wherein
  - a. when a substitution occurs at position 193, the substituting amino acid is Asn, Cys, Gln, His, Ile, Met, Thr or Tyr;
- F. when a substitution occurs in the sixth loop region, the substitution occurs at one or more of positions 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 220, 221, 223 or 224; wherein
  - a. when a substitution occurs at position 204, the substituting amino acid is Asn, Asp, Gln, Glu, Gly, His, Pro, Ser or Thr;
  - b. when a substitution occurs at position 205, the substituting amino acid is Asn, Asp, Gln, Glu, Gly or Ser;
  - c. when a substitution occurs at position 206, the substituting amino acid is Asn, Asp, Gln, Glu, Pro or Ser;
  - d. when a substitution occurs at position 207, the substituting amino acid is Asp or Glu;
  - e. when a substitution occurs at position 208, the substituting amino acid is Asp or Glu;
  - f. when a substitution occurs at position 209, the substituting amino acid is Ala, Asn, Asp, Cys, Gln, Glu, Gly, His, Leu, Met, Pro, Ser, Thr or Val;
  - g. when a substitution occurs at position 210, the substituting amino acid is Asp, His, Ile, Leu, Met or Pro;
  - h. when a substitution occurs at position 211, the substituting amino acid is Asp or Glu;

- i. when a substitution occurs at position 212, the substituting amino acid is Asn, Asp, Gln, Glu, Pro or Ser;
- j. when a substitution occurs at position 213, the substituting amino acid is Asp, Glu, His, Ile, Leu, Met, Phe, Pro or Val;
- k. when a substitution occurs at position 214, the substituting amino acid is Asn, Gln, Gly, or Ser;
- l. when a substitution occurs at position 215, the substituting amino acid is Asn, Asp, Gln, Glu, Pro or Ser;
- m. when a substitution occurs at position 216, the substituting amino acid is Asp or Glu;
- n. when a substitution occurs at position 217, the substituting amino acid is Asp, Glu or Pro;
- o. when a substitution occurs at position 220, the substituting amino acid is Asp or Glu;
- p. when a substitution occurs at position 221, the substituting amino acid is Ala, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile, Met, Pro, Ser, Thr or Val;
- q. when a substitution occurs at position 222, the substituting amino acid is Asp or Glu;
- r. when a substitution occurs at position 223, the substituting amino acid is Asn, Asp, Gln, Glu, Pro or Ser; and
- s. when a substitution occurs at position 224, the substituting amino acid is Asn, Asp, Gln, Glu, Pro or Ser;

whereby the Thermitase variant has decreased adsorption to, and increased hydrolysis of, an insoluble substrate as compared to wild-type Thermitase.

- 2. The Thermitase variant of Claim 1, wherein one or more substitutions occur in the first loop region.
- 3. The Thermitase variant of Claim 1, wherein one or more substitutions occur in the second loop region.

4. The Thermitase variant of Claim 1, wherein one or more substitutions occur in the third loop region.
5. The Thermitase variant of Claim 1, wherein one or more substitutions occur in the fourth loop region.
6. The Thermitase variant of Claim 1, wherein one or more substitutions occur in the fifth loop region.
7. The Thermitase variant of Claim 1, wherein one or more substitutions occur in the sixth loop region.
8. A cleaning composition selected from the group consisting of a hard surface cleaning composition, a dishwashing composition, an oral cleaning composition, a denture cleansing composition, a contact lens cleaning composition and a fabric cleaning composition, characterized in that the cleaning composition comprises the Thermitase variant of any of Claims 1-7 and a cleaning composition carrier; preferably the cleaning composition is a hard surface cleaning composition or the cleaning composition is a fabric cleaning composition; preferably the composition comprises at least about 5% surfactant and at least about 5% builder, by weight of the composition; preferably the composition further comprises cleaning composition materials selected from the group consisting of solvents, buffers, enzymes, soil release agents, clay soil removal agents, dispersing agents, brighteners, suds suppressors, fabric softeners, suds boosters, enzyme stabilizers, bleaching agents, dyes, perfumes, and mixtures thereof.
9. A DNA sequence encoding the Thermitase variant of any of Claims 1-7.

# INTERNATIONAL SEARCH REPORT

International Application No  
PCT/US 96/03009

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C12N15/57 C12N9/52 C11D7/42 C12N1/21 A61K7/28  
/(C12N1/21,C12R1:125)

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C12N C11D A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO,A,91 00345 (NOVONORDISK AS) 10 January 1991 see the whole document ---	1-9
Y	EP,A,0 405 901 (UNILEVER PLC ;UNILEVER NV (NL)) 2 January 1991 see the whole document ---	1-9
Y	EP,A,0 405 902 (UNILEVER PLC ;UNILEVER NV (NL)) 2 January 1991 see the whole document ---	1-9
Y	EP,A,0 380 362 (GENEX CORP) 1 August 1990 see the whole document ---	1-9
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☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

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Date of the actual completion of the international search

8 July 1996

Date of mailing of the international search report

25.07.96

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2  
NL - 2280 HV Rijswijk  
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,  
Fax (+31-70) 340-3016

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Hix, R

# INTERNATIONAL SEARCH REPORT

International Application No  
PCT/US 96/03009

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>ACTA BIOLOGICA ET MEDICA GERMANICA, vol. 41, no. 2/3, 1982, pages 137-144, XP000574173 G. HANSEN ET AL.: "Thermitase, eine thermostabile Serin-Protease aus Thermoactinomyces vulgaris: Wechselwirkung zwischen aktivem Zentrum und SH-Gruppe des Enzyms" see the whole document ---</p>	
A	<p>STUDIA BIOPHYSICA, BERLIN, vol. 72, no. 2, 1978, pages 61-67, XP000573697 P. BENDZKO ET AL.: "Untersuchungen zur Konformationsstabilität der thermostabilen alkalischen Serin-Protease Thermitase" see the whole document ---</p>	
A	<p>INT. J. PEPTIDE RES. , vol. 22, 1983, pages 66-72, XP000573692 M. BAUDYS ET AL.: "Amino acid sequence of the tryptic SH-peptide of thermitase, a thermostable serine proteinase from Thermoactinomyces vulgaris" see the whole document ---</p>	
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International Application No

PL I/US 96/03009

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